THE JOURNAL

OF

COMPARATIVE NEUROLOGY

VOLUME 27

1916–1917

CONTENTS
1916–1917

No. 1. DECEMBER, 1916

No. 2. FEBRUARY, 1917

No. 3. APRIL

No. 4. JUNE

Susanna Phelps Gage.
March 1898

THIS VOLUME OF

THE JOURNAL OF COMPARATIVE NEUROLOGY

IS DEDICATED TO THE MEMORY OF

SUSANNA PHELPS GAGE

WHOSE CONTRIBUTIONS TO COMPARATIVE NEUROLOGY HAVE WON
AN HONORABLE PLACE IN THE PERMANENT STRUCTURE OF
THE SCIENCE, AND WHOSE PERSONALITY COM-
MANDED THE ESTEEM AND AFFECTION OF
ALL HER ASSOCIATES

SUSANNA PHELPS GAGE, PH.B.

Mrs. Gage was born and spent her childhood and early youth in Morrisville, the county seat of Madison County, New York.

The father, Henry S. Phelps, was also born in Madison County, but his father, John, and grandfather, Elijah Phelps, came from New England where their ancestors were among the earliest English settlers. The grandfather was a soldier of the Revolutionary War. The father, John Phelps, died before the children had reached manhood. This made it necessary for the boy, who was to be the father of the subject of this sketch, to play the part of a man early in life.

A part of his boyhood and youth were spent in Cortland County in and near Cortland, but his mature life was lived in the village of Morrisville where he was a leading merchant, and a citizen respected and trusted by the community.

The mother, Mary Austin Phelps, was a native of Cortland County, and like the father was of New England descent, with Scottish as well as English blood. Her father was a prosperous farmer and mill owner in the rich Cortland valley and could give his children more of the comforts of life and especially the advantages of the best education then available for young women. But, as was the custom in those days, the education was not alone in books but in the real work of the household and the art of conducting a home, which could come only with a perfect familiarity with all the work which makes such a home possible. She early became a teacher, and had a genius for starting young people on the road to learning; and it was a sound start she gave them; no shirking was permitted and no slipshod work accepted. She taught several years (1847–1854) in the South, and saw that region from the inside in what seemed its most prosperous period of slavery and chivalry.

Mrs. Gage's parents were then these two people with their sound heredity and wholesome youthful training, and their rather broad experiences. Naturally they were interested in and upholders of the church, the schools, and all the other enterprises for the betterment of the community. While these interior villages of the country could not all have colleges or seminaries for higher learning, they could have the lyceum on whose stage came some of the best men of the country; and her parents did their full share in supporting this institution.

With such a father and mother it seemed perfectly natural that the child should have a sound character, and not at all surprising that there was strong love of learning and a taste for the finer things of life. While of course the father and mother wished all good things for their daughter, they were not so fatally unwise as to neglect the homely duties in education and training, for they knew full well that the finer things of life come only through the portals of labor and service; they knew also that the labor was not the only purpose of life, only a means to an end.

Morrisville, the birthplace and home, is in one of the beautiful valleys so common in New York State. The surrounding country is a rich farming region with its hills and upland plateaus. On the north not far away are Utica and Syracuse, and to the south is Binghamton. In the neighboring villages of Hamilton and Clinton are Colgate and Hamilton colleges.

The home was in the midst of the village just across the street from the school house and the churches, and the father's store was only a short walk down the street. Higher up on the street stood the court house and other county buildings. This main street was a part of the once famous Cherry Valley Turnpike. Before her eyes then as she grew up were the physical representations of transportation in the turnpike; law and government in the county buildings; education in the school house, and the spiritual life in the churches. While the village in the valley seemed in security and peace as if surrounded and protected by the everlasting hills, those same hills gave opportunity for the wide view and the call that comes from mountain tops

to the larger world, and she loved to go where she could see these wide views; and her subsequent career showed that she heard also the call to the larger world beyond the hills.

The early education was like that of practically all children in the more settled parts of New York State between 1860 and 1870,—the village school, with its variety of teachers, young men aspiring to the ministry or the law for the winter terms and young women also preparing for the real work of life in the summer. The difference, and it was a fundamental difference, lay in the fact that the mother, with her fine instincts for teaching and sound training, supplemented the regular school work and saw to it that there was a thoroughness in the elements which should lay the foundation for any attempts which time or circumstance might make possible; and in the mother's mind was the hope for some college training such as women were just coming into the possibility of having at that time.

As the years advanced and the eager zest of the young woman for learning and the better things of life manifested themselves with her growth, the college decided on for her was Vassar, and preparation for entrance was undertaken at the then famous Cazenovia Seminary. There she came in contact with some of the high-minded advanced teachers who gave uplift in those days to so many young men and women and showed the beauty and the possibilities in the intellectual and spiritual life. She was especially inspired by the teacher of Latin, Isaac N. Clements, later the head of the school. This man had had the stern training of the Civil War and knew life and its savagery as well as the blessed side represented by the gospel, of which he was one of the ministers. In the home of his one-time pupil he recently told the husband and son of the enthusiasm, fullness of life, and intellectual vigor of his former student.

As stated above, Vassar College had been in mind for the young woman and was the choice of the mother. But the father had been stirred by the accounts he had heard of Cornell University. The father especially could understand and appreciate the splendid promise of the new institution and was captivated by the dreams of Ezra Cornell for the education of the

young men and women of the country; and the broad scope of
that education as outlined by Mr. Cornell and President White
appealed to him who knew from his own experience in the world
the need of something in addition to a knowledge of the ancient
classics. Henry W. Sage had just built and given Sage College
for housing the women students of the University so that pro-
tection as well as education seemed cared for. The father who
had known almost pioneer life did not hesitate because the
institution was young, and perhaps a little rough; he knew by
experience the fundamental virtues residing in youth and
roughness. His wishes, aided by the adventurous spirit of the
daughter, prevailed, and he came with her to the University
which she entered in the autumn of 1875. As good fortune
would have it, when their carriage drove up to the entrance of
the newly finished Sage College, President White was there.
For as was his custom, he had been looking at this building,
as he always did at all growing buildings, to see if it was all
ready for the fine young women he felt sure would come to it
to receive the instruction already given men. He welcomed
the father and daughter and went into the building with them
and saw to it that food and a room were provided for the first
Sage College student.

The course pursued in college included a further study of
Latin, much English literature, and a large amount of historical
study. In the historical study two subjects seemed of para-
mount interest, Roman history and American constitutional
history. She also never failed to make the most of every op-
portunity to hear the lectures of Goldwin Smith on English his-
tory, and those of Andrew D. White on modern European history.

With these classical, literary, and historical studies came
studies in modern science, among which physics and biology
took the strongest hold upon her. She was the first woman to
take laboratory work in physics in Cornell University. The
facilities for laboratory work were limited, the only space being
under the raised seats of the lecture room. But as with all her
other teachers, Prof. Wm. A. Anthony, the founder of the de-
partment of physics at Cornell, believed so much in the ability
and earnestness of his aspiring pupil that he gave her space and

opportunity for laboratory work in his own cramped quarters. Later he was often a guest in her home and seemed to rejoice at the sacrifice he had made to give her opportunity to work out for herself some of the fundamental things in physics. In passing it may be said that she never had any trouble in convincing her teachers that it was worth while giving her a chance to do things. The animated face and honest gray eyes kindling with enthusiasm were her passport everywhere.

It was not to be in physics that she was to do her intellectual life work, however, but in the zoological side of biology. The teaching staff in zoology and comparative anatomy at that time was presided over by Prof. Burt G. Wilder for the vertebrate side, and Prof. J. H. Comstock for the invertebrates, in which entomology was the major interest. She took all the courses offered by the two departments.

In 1881 occurred her marriage to Asst. Prof. Simon Henry Gage. This gave opportunity for work and investigation in biology. She made the most of her chance, entering at once with full enthusiasm into the work of her husband, making drawings for his papers, and wall diagrams for his courses, and many also for Professor Wilder's courses. But the naturally independent mind could not long be satisfied as a mere helper; there was a desire to undertake some original work on her own account.

At that time the form and relations of the fibers of striated muscles were not well understood, and especially were they misunderstood with small animals like mice and small birds. So it became her first published scientific work to show what the relations of the fibers really were in the small animals and later in laboratory animals generally. So fundamental and convincing was her work, and so clear the drawings accompanying the text that the veteran Kölliker revising his Histology for the sixth time, declared in his introduction that the literature is now so large that he can only refer to the 'Allergewichte,' and gave her as authority for the statement with which he closes the discussion of the form and length of the fibers in striated or skeletal muscles (Kölliker's Gewebelehre, Sechste Auflage, Bd. I, p. 371). And in a letter from Dr. Minot who was giving some

lectures in embryology at Cornell he says, concerning the muscle work: "I was greatly interested in your wife's muscle preparations which are convincing as to the great accuracy of her observations."

As shown by the list of her publications at the end, her independent work covered a considerable range, and she joined in the preparation of papers upon a variety of subjects. But her main work was in neurology, for as a student, and especially as a member of Prof. Burt G. Wilder's seminary, the nervous system grew in fascination for her. It is not known by many, perhaps, that as assistant to Louis Agassiz, Dr. Wilder was urged by that great man to interest himself in the nervous system. Finally the wisdom of Agassiz' advice appealed strongly to him and as early as the college year 1870–1871, Dr. Wilder gave a special course in comparative neurology. In 1875 this course in neurology became fully established in Cornell. As stated in the introduction to the Wilder Quarter Century Book: "It is in this course of neurology perhaps more than in any other that is realized [in him] the picture drawn by Agassiz, in his address at the inauguration of Cornell University, of the teacher going before his class with his own thoughts and as an elder brother inspiring his pupils to the most enthusiastic effort."

Certain it is that the inspiration for her was masterful, and with growing maturity of thought, the phylogeny, development and morphology of the nervous system was to her the supreme question in biology.

Of her twenty-six independent publications, ten were upon neurology. These were her most important papers and they comprised 65 per cent of the total number of pages written by her. An estimate of the value of her papers would hardly be in place here. It may be said, however, that her writing has a clearness and directness that make her meaning unmistakable. She did not hope to have all agree with her, but she did hope that all could understand exactly what she meant. She had no patience with the oracular form of writing which could be interpreted in almost any way, and to which discoveries made long afterward could be referred.

From her own standpoint, no pains were too great to make sure that the interpretation she had made of structure or of ideal plan of structure and relationship were the true ones. As with all investigators endowed with the divine gift of imagination, some of the cherished images had to be broken when the facts discovered on fuller study showed that they were false, but never was an iconoclast more merciless than she when once convinced that the images were false. Furthermore, her work won her the respect of her fellow investigators in her own and in other countries. She was welcomed in all biological laboratories and given every facility. No single laboratory can hope to be adequately equipped in embryologic and anatomic series of all forms and stages and prepared by all the standard methods; hence the need of making use of the facilities of many laboratories to gain the broadest view on some fundamental questions. Fortunately there is the spirit of helpfulness in the laboratories, and any worthy worker is given the needed facilities without stint. She was freely accorded such facilities. Most often she used the rich collections of Dr. Minot at the Harvard Medical School, and of Dr. Mall at the Johns Hopkins Medical School. Indeed, one of her most important papers was based on a three weeks embryo loaned to her for more than a year by Dr. Mall.

In 1911 it became possible for her to carry out a long hoped-for visit to Europe to see the places where history had been made, to enjoy the art, and most of all, to see some of the laboratories from which have originated so much of the fundamental work in modern science. Fortunately the meeting of the Anatomische Gesellschaft was held soon after her arrival in Leipzig and the British Association for the Advancement of Science a few months later. These meetings gave her a chance to see with her own eyes the methods and work of two of the great foreign societies, and to compare them with those of the societies of her own country with which she was so familiar.[1] She became a member of the German Anatomical Society; and

[1] Mrs. Gage was a fellow of the American Association for the Advancement of Science, a member of the American Anatomical Association, the American Society of Zoologists, the American Microscopical Society and the Anatomische Gesellschaft.

later in visiting some of the universities the distinguished men whom she had met showed her every courtesy in their laboratories and gave her opportunity to study the choicest among their series. Among the experiences most cherished were those connected with the laboratory of Golgi in Italy and of Ariëns-Kappers in Holland. Golgi not only showed her some of the specimens already mounted, but made for her before her own eyes some of his incomparable preparations.

In speaking of college studies above, it was stated that among those which gave her the greatest pleasure and inspiration was American history; and a few years later while staying with her father during a period of family bereavement, she read aloud to him Irving's life of Washington. She was keen in perceiving the breadth and foresight of Washington in all educational matters and was especially interested in his desire for a national university, for the founding of which he gave a generous share of his private fortune.

Her own intimate knowledge of the need for research opportunities in our country, if it were to become a real and a wise leader among the nations of the earth, led her to ponder deeply on such an institution, the crown of our educational system, which has not yet been realized in our land. It seemed to her that the women of the nation, who were coming into the sunlight of opportunity in university education, if they only knew of this great hope and desire of Washington and the urgent need, would rally with the same enthusiasm as that which filled her mind and heart and the greatest of all our American universities would arise in Washington City—an institution which would represent the highest and best in the university idea, and which, being the offspring of the whole country, would realize Washington's dream and hopes far beyond what he could have imagined.

She helped to found the George Washington Memorial Association to bring about the hoped-for result, and served it for several years as secretary. From 1895 to 1905 she urged in spoken words and in writing the fulfilment of this dream of Washington with a zeal and eloquence worthy of success. Perhaps her

point of view may best be seen by a quotation from her paper before the University Convocation held in the senate chamber at Albany in 1897. The subject of the paper was the need of a national university for the common schools, and the quotation follows the description of the vitalizing power with which a university teacher had conducted a common school exercise in a difficult subject.

But we can not spare from their present work of investigation the few men and women in the country who are specialists, to preach their gospel in the highways and hedges. We need thousands of young men and women who shall go to these specialists and receive such training that they can give living inspiration to the children. We need more and more places where this supreme training can be acquired, we need finally a national university where in quiet thoughtfulness the newest questions may be solved and the oldest be reconsidered. The knowledge gained here at the very surface of the known, will then penetrate through college and high school and common school to the living units of the great democratic mass, that each may live a life richer in the things of the spirit. He at the very outside not only gives to him within but some day he helps the pupil now standing at his side to mount upon his shoulders and push a little farther into the hitherto unknown universal knowledge.

Like so many of the splendid dreams of noble men and women she did not see even the beginnings of a National University realized any more than did Washington. Apparently it must wait, but that it would come true sometime she never doubted.

While the great public service she hoped to render in helping to make real Washington's plan for higher education seemed to fail, she found it possible to aid her native village by the gift to it of her childhood home for a library. And now in her father's house is one of the best libraries of any village in the state; and it is greatly appreciated by the people of the surrounding region as well as by the villagers.

In the bookplate and the bronze tablet which she designed for the library, the bittersweet was used for decoration. This came about naturally from the fact that for many years a magnificent bittersweet vine was a distinguishing feature of her father's house, and the clusters of red fruit gave it in winter the appearance of cheer that the abundant flowers around the house

lent in summer. In the bookplate is this sentiment going with the decoration and expressing what books had been to her: *"The bitter and the sweet of all the past shall strengthen us."*

Doubtless as the years pass away many a boy and girl will find in some book in that library the intellectual and spiritual food which they long for and which will show them the way they are seeking to a career of noble living, and of service to their day and generation comparable with that of the gracious woman who made the library possible.

It was always a pleasure to her to attend the seminaries in the different biological groups in the university and there was ever a word of commendation and cheer for the young workers who were trying to walk alone. They loved to talk over their work and plans with her, and none ever did so without having his purpose strengthened.

It was not only in the laboratories and seminaries that she met the students, but they were welcomed in her home. And from the testimony of many who have found high success those home experiences added to life and its aspirations what could not be given by the laboratory alone. She was much sought after not only by the students but the young people in the teaching staff of the university who found in her the abounding sympathy and enthusiasm of youth combined with the wisdom that comes only from maturity and experience; and none who sought help ever went away empty handed but all gained from her new courage and enthusiasm, new faith that life was worth living, and that something worth while could be accomplished in the world.

As expressed by her friend of thirty-five years, Mrs. Anna Botsford Comstock, in the Cornell Alumni News of November 4, 1915:

Mrs. Gage's personality made a lasting impression upon all who met her. She had great charm of manner, deep earnestness, a vigorous and quaint originality of thought and expression, a fine sense of humor, keen sympathies, and above all the power of bringing cheer to all with whom she came in contact. Her merry musical laugh was so much a part of her that even those of us most bereft must be comforted by the memory of it. Her character gave a firm and broad basis

for her attractive personality. She had high ideals, unswerving honesty and singleness of purpose, and great power of helpfulness to the person or cause that might be in need. She had a genius for friendship for her heart was loyal and loving. Her strong mental fiber and keen intellect rendered her friendship as stimulating as it was comforting.

On Saturday, Sunday, and Monday, the 2d, 3d, and 4th of October, 1915, the son, Henry Phelps Gage, Ph.D., visited the home and during that time explained and demonstrated to his mother the daylight glass he had succeeded in producing, and showed its use for microscopic work in demonstrating the most delicate colors and tints equal to what could be accomplished by daylight, and also the use of a large lamp with the daylight glass for matching delicate colors of silks, and for water color work. What parent is there who cannot imagine the happiness that came to the mother's heart to see realized in the accomplishment of her son some of the dreams that the long-before laboratory work in physics of her own youth gave rise to.

On Sunday there was an automobile ride along Cayuga Lake and on the return was one of the gorgeous sunsets that Ithaca is famed for. We did not know then as we gazed upon it that one of our number was so soon to enter into that glory.

After four years of failing health, death came suddenly and painlessly on the morning of October 5, 1915.

In the home at Ithaca were simple funeral services, a part of which were some of the heart-sustaining and soul-uplifting hymns she loved so well, played upon the university chimes.

As long mutually agreed upon, the body was cremated; and in the childhood home which she had given for the village library, with the books looking down from the shelves, and in the presence of life-long friends, some fitting words were spoken over the ashes. These now rest in the village cemetary beside those of the father and mother and brother who had preceded her.

The sky that she looked up to with such joy in childhood looks down upon the quiet resting place. The encircling hills, from which in youth she looked forth with such enthusiasm and high courage to the world of work and service, shall hold forevermore their guardianship over the beautiful valley.

PUBLICATIONS OF SUSANNA PHELPS GAGE

1880 The commonwealth of mind. The Cornell Review, June, vol. 6, pp. 346–351. This paper was given as class essayist at the graduation of her class in 1880. It is an argument and an appeal for the fundamental democracy of the mind in human beings, and this remained her cherished belief throughout life.

1887 Ending and relation of the muscular fibers in the muscles of minute animals (mouse, mole, rat, and English sparrow). Abstr. Proc. Amer. Soc. Micro, pp. 1–2.

1888 Form, endings, and relation of striated muscular fibers in the muscles of minute animals (mouse, shrew, bat, and English sparrow). The Microscope, vol. 8, August, pp. 225–237; 257–272, 5 pl. It is in these two papers that the author expounds the true form and relationship of the fibers of skeletal muscle in small animals.

1890 The intramuscular endings of fibers in the skeletal muscles of the domestic and laboratory animals. Proc. Am. Soc. Microscopists, 13th meeting, pp. 132–138, 1 pl.

1891 A review. The evolution of sex, by Prof. Patrick Geddes and J. Arthur Thompson. The Nation, vol. 52, May 14, p. 407.

1904 The story of little red-spot. Boys and Girls, April, pp. 11–16, 1 fig. The author says of this story: "The scientific name of Red-Spot is Diemyctylus viridescens . . . . The photoengraving on p. 11 is taken from the colored lithographic plate drawn by the present author for an article by Prof. S. H. Gage in the American Naturalist, December, 1891, where he tells the same story in scientific language. The present author when making the drawings studied red-spots in their homes and wrote the story for her little son." Anna Botsford Comstock, the nature study expert, says: "It is one of the most charming science stories for children in our literature."

1892 Evolution and the training of children. Abst. Kindly Light, vol. 1, no. 6, p. 3, April.

1892 A reference model. Proc. Amer. Soc. of Micros. (Rochester meeting), pp. 154–155, 1 fig, vol. 14, 1892, printed July, 1893.

1893 The brain of Diemyctylus viridescens from larval to adult life, and comparisons with the brain of Amia and Petromyzon. Wilder Quarter Century Book, pp. 259–313, 8 pl.

1895 . . . . with Anna Botsford Comstock, editors and authors. A tribute to Henry W. Sage from the women graduates of Cornell University. Ithaca, N. Y., May 30, 84 pp. Illustrated by Anna Botsford Comstock. This is the fullest and best discussion of co-education in a great university, and the only adequate account of it in Cornell University.

1896 Lines on the engraving "Two incarnations in stripes," by Anna Botsford Comstock. Illustrated Buffalo Express, March 1, p. 5.

1895–1896 Comparative morphology of the brain of the soft-shelled turtle (Amyda mutica) and the English sparrow (Passer domesticus). Proc. Amer. Mic. Soc., vol. 17, pp. 185–238, 5 pl. Abstr. Am. Month. Mic. Journ., vol. 17, Jan., pp. 4–7.

1896 Modification of the brain during growth. Amer. Assoc. Adv. Sci., August 24, 1896. See Proc. Abstr., Amer. Naturalist, October, pp. 836–837. Science N. S., vol. 4, October 22, 1896, pp. 602–603.

1896 The brain of the embryo soft-shelled turtle. Trans. Amer. Mic. Soc., vol. 18, pp. 282–286.

1897 Washington and the national university. The New Unity, June. Reprinted, Active Interests, December, 1897, pp. 15–23. With bibliography and plea for George Washington Memorial, pp. 6–7.

1897 The need of a national university in its relation to the common school. Proc. 35th University Convocation (Albany), June, pp. 313–319.

1898 A Washington memorial university. The Outlook, February 26, pp. 521–524.

1898 Relation of a national university to the graduate departments of existing universities. Address given at a meeting of the George Washington Memorial Association, December 15, pp. 15–27. Papers of 1897 and 1898 cannot be read without admiration for her breadth of view and grasp on the educational conditions of our country. Her devoted patriotism and sympathy with the ideals of Washington are shown in every paragraph.

1899 Notes on the chick's brain. Abstr. Amer. Assoc. Adv. Sci. Proc., vol. 48, p. 256.

1902–1903 An unusual attitude of a four weeks human embryo. Comparisons with the mouse. Abstr. Proc. Am. Assoc. Adv. Science, vol. 52, p. 458. Science N. S., vol. 17, 1903, p. 254.

1904 The mesonephros of a three weeks human embryo. Proc. Assoc. Am. Anat., March, p. VI. in Am. Jour. Anat., vol. 3, 1904.

1904–1905 Total folds of the forebrain, their origin and development to the third week in the human embryo. Proc. Assoc. Amer. Anat. in Am. Journ. Anat., vol. 4, no. 2, 1905, p. IX.

1905–1906 Relations of the total folds of the brain tube of human embryos to definitive structure. Proc. Assoc. Am. Anat., 20th Ses. in Am. Jour. Anat., vol. 5, pp. IX–X.

1905 A three weeks human embryo with especial reference to the brain and the nephric system. Am. Jour. Anat., vol. 4, no. 4, pp. 409–443, 5 pl. The embryo for this study was loaned by Dr. F. P. Mall, and he expressed himself satisfied with the results gained from its study. This is one of Mrs. Gage's most important papers, and illustrates well her method and thoroughness of work.

1906 The notochord of the head in human embryos of the third to the twelfth week and comparisons with other vertebrates. Abstr. Proc. Am. Assoc. Adv. Sci., vol. 56, pp. 277–278. Science N. S., vol. 24, September 7, 1906, pp. 295–296.

1907 The method of making models from sheets of blotting paper. Anat. Record, no. 7, of Am. Jour. of Anat., vol. 7, no. 3, November 10, pp. 166–169. Read, Assoc. Am. Anat., December, 1905. Abstr. Am. Jour. Anat., vol. 5, 1905–1906, p. XXIII, Demonstr, 7th Internat. Zool. Cong., August, 1907. These models combine the good features of the French papier maché and the German wax-plate models, and represent her inventive turn of mind, and ability to adapt means to ends.

1907   Changes in the form of the forebrain of human embryos during the first
       8 weeks.   Read, Seventh International Zool. Cong., August.   Printed
       in Proc. Seventh International Zool. Cong., 1910, 2 pp. 3 figs.

PAPERS IN COLLABORATION WITH S. H. GAGE

As stated in the text above, Mrs. Gage entered enthusiastically into the origi-
nal work in biology and for a long time made most of the drawings to illustrate
her husband's papers.   She also became partner, in a broader sense, in the papers
named below:

1885–1887   Aquatic respiration in soft-shelled turtles (Aspidonectes and Amyda).
       A contribution to the physiology of respiration in Vertebrates.   Proc.
       Amer. Assoc. Adv. Sci., vol. 34, Ann Arbor meeting, August, 1885,
       pp. 316–318.   Amer. Naturalist, 1886, pp. 233–236.   Science, Septem-
       ber 11, 1885, p. 225.   Scientific Amer. Supplement, to November 14,
       1885, p. 8230.   Biologisches Centralblatt, Bd. 6, 1886–1887, pp. 213–214.
1886   Amœboid movements of the cell-nucleus in Necturus.   Science, vol. 7,
       p. 146.
1888   Combined aerial and aquatic respiration.   Science, vol. 7, 1886, p. 394.
       See note in Ref. Handbook Med. Sci., vol. 6, p. 197.
1886   Pharyngeal respiratory movements of adult Amphibia (Diemyctylus)
       under water.   Science, vol. 7, p. 395.
1889   Staining and permanent preservation of histological elements isolated by
       means of caustic potash (KOH) and nitric acid (HNO₃).   Proc. Amer.
       Soc. of Micr., vol. 11, pp. 34–45.   Gage (Dr. Simon H., et Suzanne P.).
       Coloration et conservation permanentes des éléments histologique
       isolés par la potasse ou l'acid nitrique.   Jour. de Micrographie, t. 15,
       1890, pp. 43, 102.
1890   Changes in the ciliated areas of the alimentary canal of the Amphibia
       during development, and the relation to the mode of respiration.
       Abstract, Proc. Amer. Assoc., Adv. Sci., vol. 39, pp. 337–338.
1908   Sudan III deposited in the egg and transmitted to the chick.   Science,
       N. S., vol. 28, pp. 494–495.
1909   Coloration of the milk in lactating animals and staining of the growing
       adipose tissue in the suckling young.   The Anat. Record, vol. 3,
       April, pp. 203–204.
1898   The life history of the toad.   Teachers Leaflet, No. 9, for use in the public
       schools, pp. 79–98, 8 figs.   Prepared by the College of Agriculture,
       Cornell University, I. P. Roberts, Director.   Issued under Ch. 67,
       Laws of N. Y., 1898.   2d revised ed., 1904. pp. 185–206, 14 figs.   (In
       bound volume of Cornell Nature Study Leaflets.)   Abbreviated by
       Susanna Phelps Gage from Leaflet No. 9, Series G.   Nature Study
       and published by the League for Social Service of N. Y. City, Geo.
       Leighton Tolman Donation.   Spring of 1899.   The Sanitary Home,
       vol. 3, May, 1901, p. 57+.

THE CEREBRAL GANGLIA AND EARLY NERVES OF SQUALUS ACANTHIAS

F. L. LANDACRE

Department of Anatomy, College of Medicine of the Ohio State University

THIRTEEN FIGURES

CONTENTS

19

1. INTRODUCTION

Up to the present time no detailed analysis of the cerebral nerves of the shark has been published. A voluminous literature covering almost every other phase of the anatomy and embryology exists, but for some reason an analysis of the cerebral nerves of the shark such as we now have for a number of fishes and amphibians and reptiles does not exist. The present study of the embryonic ganglia and early nerves is not offered, as, in any sense, a substitute for such an analysis of specimens old enough to show complete medullation of the nerves. The author has attempted an analysis similar to those made on Ameiurus ('10), Lepidosteus ('12), and Rana ('12), in which it was found that there was a particularly favorable condition of the ganglia in that they were well isolated, and a development of the chief nerves sufficient to enable one to identify them with certainty when their composition was known in detail in the adult.

The present study has the disadvantage of not being preceded by a careful analysis of older specimens but, like the previous studies mentioned, has the advantage of presenting a very simple condition of the various ganglia and, in cases where the nerves are pure or contain only one component, the morphological relations of the ganglia and nerves make their identification a simple matter. On the other hand, mixed nerves and very immature nerves present greater difficulties and in these cases where the various components could not be traced definitely to their distribution they have been identified provisionally.

The amount of attention given to the description of nerves in a paper devoted ostensibly to the description of ganglia would be unwarranted if the exact composition of the nerves were known. In the absence of this information the nerves had to be followed with the greatest care and their description is in-

cluded in the body ot the paper. The general morphological relations of ganglia are fairly safe criteria for their identification provided one is sure of their presence; otherwise, the distribution of the fibers must be known to identify a ganglion with certainty.

The differences in size in the cells of different ganglionic components sometimes found in other types and particularly in mature types do not seem to exist in the material studied; such differences as exist are those between older and younger ganglion cells rather than between different components. However, I do not believe any serious oversight has been made unless it is in the failure to find a general cutaneous component in the seventh and ninth nerves.

The number of nerves described is small compared with the adult, of course, but those present in the stage described are the chief nerves. While this study should have followed and not preceded that of older material, it is hoped that it will help to fill the gap in our knowledge of our most generalized vertebrate and it will certainly serve as a foundation for the study of the origin of the cerebral ganglia on a component basis. The effort to describe the origin of these ganglia, en masse, is entirely futile in the author's opinion. It must be done with a thorough knowledge of all the ganglia involved. This is true whether they arise as discrete ganglia having different sources of origin or whether they differentiate out of a common primordium.

The author is under obligation to Dr. H. V. Neal for most of the material, which was fixed in vom Rath's fluid and mounted unstained. This material was supplemented by younger material stained in Delafield's haematoxylin and orange G. The embryos ranged in length from 18 to 30 mm. after fixation and several specimens of each length, except the 30 mm. embryo, were examined. The plot and drawings were made from a 22 mm. embryo. The terms anterior, posterior, dorsal and ventral are used in the body of the paper to indicate the relative positions of structures on the plot and not their true position in the adult which is sometimes quite different.

2. THE NERVUS OLFACTORIUS AND NERVUS TERMINALIS

The connection between the olfactory pit and forebrain, which has no olfactory bulb at this time, consists of a thick cellular and fibrous mass which probably represents both the above mentioned nerves. The main portion of this connection, that lying more dorsal in position in the plot (fig. 1, *n.olf.*), consists of a dense mass of medium sized cells extending from the brain wall back to the epithelium of the olfactory capsule, where, just before coming into contact with the brain, it breaks up into three or four masses of cells where it is connected with the brain. The anterior end of this mass is solid and does not show the loose character of the posterior end. There are isolated strands of fibers in this mass aside from those mentioned below but their connection with neither the brain wall nor the olfactory epithelium could be made out with certainty. These strands of fibers are identified as olfactory fibers.

In addition to the main dorsal portion described above, there are two strands of cells on the anterior end of the nerve lying ventral to the main strand containing definite fiber bundles (fig. 1, *N.Ter.*). The entrance of these fibers into the brain wall at a point more ventral and median than the main mass of cells is easily made out and the fiber bundles can also be traced through the connecting cellular mass to the olfactory epithelium. These two strands in the specimen plotted are represented by only one strand in four other specimens of about the same age and there is only one strand of cells and fibers on the opposite side of the same specimen. Both strands are accompanied by a limited number of round cells lying in the position indicated by Locy ('99, '05) as the location of the ganglion of the nervus terminalis, namely near its entrance into the brain wall. In the 30 mm. embryo the ganglion of the n. terminalis while small is well defined and well isolated. There can be no doubt as to the identification of these as the nervus terminalis on account of the point of entrance into the brain wall and the greater degree of development. Locy ('99, '05) gives a rather full account of the history of this nerve, but his account shows

both the olfactory and terminalis nerves to be much more developed and better isolated at 25 mm. than in my specimen of the same age.   In fact, one would infer from his description that at 16 mm. the connections of the olfactory and terminalis nerves were more definite than in the 22 mm. embryo plotted in this paper.

At the posterior end of the connecting mass in all specimens examined there are two strands of cells which detach themselves from the main mass and come into contact with the olfactory epithelium at a point more ventral and posterior to the chief mass.   These masses in none of my earlier specimens contain fibers and the strands are absent in a 30 mm. specimen.

This connecting mass appears to be, on first examination, in a rather undifferentiated condition as indicated by the small proportion of fibers, and the very large number of cells.   Another interpretation however is possible, namely, that the large mass of cells represents the beginning of the close fusion between the olfactory capsule and olfactory bulb characteristic of Squalus in addition to early sheath cells of the n. terminalis and n. olfactorius   The olfactory capsule contains at all points except the anterior border a well defined basement membrane. At the anterior border this membrane is lacking and the cells of the capsule mingle with those of the mass of cells connecting the capsule with the brain wall.   Younger embryos, in which the connecting mass is not so large, present the appearance of a migration of cells from the capsule to what I have designated the connecting mass.   However, my series of embryos is not sufficiently large to determine definitely the origin and fate of this mass of cells.   It seems from a comparison with the conditions in Amia, Ameiurus, and Lepidosteus (Brookover, 1908, 1910, 1911) entirely too large to be the ganglion of the nervus terminalis and I am inclined to interpret it as the beginning of the fusion of the capsule with the bulb plus early sheath cells, as indicated above.   The connecting mass is much smaller in younger embryos and shows a decided increase in size in a 23 mm. embryo as compared with the 22 mm. embryo plotted.

3. THE PROFUNDUS GANGLION

The profundus or mesocephalic ganglion (figs. 1 and 2, *G.Pro.*) lies just posterior to the mid-dorsal border of the eye. It is slightly crescent-shaped with the convexity on the dorsal surface. The root of the ganglion extends dorsally and slightly caudad until it comes into contact with the anterior surface of the Gasserian ganglion. The root from the ganglion to the point of contact with the Gasserian contains a rather large amount of cells and the relations of the fiber bundles are not easy to determine definitely. There seems to be little doubt, however, that the fibers from the profundus ganglion run on the anterior surface of the Gasserian ganglion, curve forward and upward and enter the brain wall through the most anterior of the three roots shown in figure 1 (*Rt.Pro.*). The point of entrance is just opposite the dorsal border of the spinal V column, which they enter.

The relations of the remaining two divisions of the portio minor are not so clear. The second division does not enter the descending V tract but enters slightly mesial to that tract and as a well isolated bundle of fibers passes to a more mesial position. This root in the specimen plotted is evidently a visceral motor root and presumably comes from the motor component of the ramus mandibularis V after running through the Gasserian ganglion. The third root in the portio minor is sensory and enters the spinal V tract. An examination of a number of other specimens ranging from 18 mm. to 23 mm. shows that there are in the older specimens several more small roots in the portio minor, all of which seem to be sensory, since they enter the spinal V tract. In all of the specimens there are two roots constantly present, the most anterior (the one identified as the root of the profundus), and the second of the three plotted in figure 1, which is identified as the visceral motor root of V. In the younger specimens these two roots are the only ones present, so that the remaining roots of the older specimens and the third root of my plot are in all probability accessory sensory roots of either the profundis or of the Gasserian, but from which ganglion they come I am unable to determine.

Neal ('98, p. 233) has discussed the relation of the various divisions of the portio minor and my results agree substantially with his rather than with those of Mitrophanow, '93. The mode of entrance of the profundus root in Lepidosteus (Landacre, '12) reinforces the identification of the most anterior root as that of the profundus since in Lepidosteus the root of the profundus enters well forward and entirely distinct from that of the Gasserian. There can be little doubt in my opinion that the second root is visceral motor. In Neal's paper he does not describe three roots in detail but figures them (Neal, '98, p. 234, fig. K.).

From the anterior end of the ganglion in the specimen plotted extends a mass of cells from which no fibers pass out, the ramus ophthalmicus profundus leaving the ganglion near its mid-ventral border. This forward extension of the ganglion is evidently the remains of the structure which Neal identifies as a persistent connection of the ganglion with the ectoderm (Neal, '98, p. 234, fig. K.) and which Scammon ('11, pp. 54, 55, figs. 11 and 12) identifies as the utrochlea process, i.e., the remains of the connection of this ganglion with the neural crest. This process gives the profundus ganglion a curious shape in contrast with nearly all other ganglia, in which the nerves practically always arise from the free end of the ganglion. In this case, as mentioned above, the profundus nerve arises near the mid-ventral border of the ganglion. The proximal portion of the profundus nerve is difficult to follow in the 22 mm. embryo on account of its being compressed between the mesial wall of the orbit and the primordia of the eye muscles and adjacent blood vessels. After it reaches a point at the level of the dorsal border of the lens its course is easily followed. It forms a gentle curve cephalad and ventral, more than half of its course being mesial to the eye. In a 30 mm. specimen the whole course of the nerve is well isolated.

The profundus nerve, aside from its large size and length as compared with the r. oph. sup. V, has the relation usual in elasmobranchs and ganoids. Only two small twigs (figs. 1, *Pro. 1*, *Pro. 2*) seem to be given off before the nerve reaches its most

peripheral point of distribution, which is to the skin at a point ventral to the point of entrance of the olfactory nerve into the brain wall. All these run to the ectoderm. The size of this nerve in the specimen plotted as compared with the r. oph. sup. V is much more like the 18 mm. embryo plotted by Scammon ('11) than the 20 mm. embryo plotted by the same author. In the 20 mm. embryo plotted by Scammon the r. oph. sup. V is much longer than the r. oph. prof. V.

4. THE GASSERIAN GANGLION

The Gasserian ganglion (figs. 1 and 3, *G.Gass.*) is very large and lies just posterior to the dorsal half of the eye. It is placed diagonally in the head with its long axis nearly in the transverse plane but with its proximal end slightly anterior to its distal end. It extends ventrally and slightly caudad from its proximal end so that its distal end lies at the level of the dorsal border of the lens. It comes into contact on its dorsal and anterior surface with the root of the profundus ganglion and on its ventral and lateral surface with the dorsal lateralis ganglion of VII. On its mesial surface it comes into contact with the m. rectus externus of the eye. Viewed from the lateral surface the ganglion is partly concealed by the dorsal lateralis ganglion of VII and by the r. oph. sup. VII and r. buccalis VII.

The ganglion is forked at its distal extremity where the two chief rami arise, but its form is not modified by the exit of the r. oph. sup. V. It is of nearly uniform thickness throughout its length. Excepting the two small roots entering with the portio minor mentioned above, the fibers passing proximally from this ganglion form a single compact and relatively massive root whose fibers pass into the spinal V tract.

5. RAMUS OPHTHALMICUS SUPERFICIALIS V

Of the three rami arising from the Gasserian ganglion at this stage, the most anterior one, the r. oph. sup. V (figs. 1 and 2, *R.O.S.V.*), is much the smallest. It arises on the anterior surface of the proximal end of the ganglion slightly dorsal and

lateral to the point of contact of the root of the profundus with the Gasserian. From its point of exit it pursues a course cephalad parallel to the longitudinal axis of the body and slightly ventral and mesial to the r. oph. sup. VII and parallel with that nerve to a point approximately over the anterior end of the lens, where it comes into contact with the superior oblique muscle of the eye. This contact is at the point of entrance of the trochlear nerve into this muscle and the sensory nerve could not be traced beyond this point in the specimen plotted, consequently no mass of cells at the growing point of the nerve could be identified such as Neal ('14, plate 7, figs. 55 and 56) figures.

There is, however, a mass of cells apparently not belonging to the muscle but slightly detached from it and containing a few large cells which may be the mass figured by Neal but seems rather to be the primordium of the sympathetic ganglion. This nerve is evidently in a much less mature condition than in the 25 mm. specimen figured by Neal. It may be mentioned incidentally that in my specimen the trochlearis does not show the two well defined rami which he figures in plate 7, figures 54 and 55. These two terminal rami in the specimen plotted are quite small. Otherwise my findings agree with those of Neal and I have nothing to add to his very thorough description of the eye muscle nerves. The r. oph. sup. V gives off one small twig about the middle of its course which runs close to the ectoderm but could not be traced into it with certainty. There is, of course, owing to the small size of this ramus no anastomosis with r. oph. sup. VII, as in the adult.

The small size of the r. oph. sup. V in Squalus as compared with the large r. oph. prof. at this stage furnishes a basis for an interesting phylogenetic comparison of these nerves, in embryos of different types. In Lepidosteus at approximately the same stage (Landacre, '12, fig. 1) the two nerves are equal in size. In embryos of urodeles (Coghill, '16, figs. 1 to 4), the ophthalmic ramus comes from the profundus ganglion and is identified by him as r. oph. prof. In Anura (Landacre and McLellan, '12) only one ophthalmic ramus comes from V and this comes from the profundus portion of the fused profundus and Gasseri-

an ganglion. Apparently the ophthalmicus profundus has supplanted largely the r. oph. sup. V in Amphibia. In higher forms the ophthalmic nerve seems to come from the Gasserian ganglion, in which case the r. oph. sup. V. has supplanted the r. oph. profundus.

6. RAMUS MAXILLARIS V

The ramus maxillaris V (figs. 1 and 4, *R.Mx.V*) arises from the ventral end of the Gasserian ganglion and pursues a course directly ventral to the position of the third lateral line organ innervated by the r. buccalis VII, where it gives off a number of twigs to the ectoderm. From this point it turns slightly cephalad and before reaching its most distal point of distribution gives off several small twigs (fig. 1, *S.1–5*), all of which run to the ectoderm. The extreme end of this nerve breaks up into a number of twigs which could not in some cases be traced to the ectoderm and present the appearance of the tip of a growing nerve.

The r. maxillaris is accompanied throughout its whole course by the r. buccalis which lies more lateral in position, but the two nerves are quite separate except at the level of the third lateral line primordium mentioned above where there are two fibrous connections between the two nerves (not shown in fig. 1) in which the fibers seem to pass from the r. maxillaris to the r. buccalis. The R. Mx. V. seems to be purely general somatic sensory. It has no connection at any point with the ganglion or nerves of visceral VII. There is a possibility that lateral line fibers enter it through the two anastomoses mentioned above, in which case it would contain a few lateral line or special somatic fibers. The appearance of the anastomoses do not favor this view and there are no lateral line primordia on the course of the r. maxillaris beyond the anastomoses. There is, further, an anastomosis (fig. 1, *R.Com.*) between the r. mandibularis V and the r. maxillaris V which will be described under the r. mandibularis V.

7. RAMUS MANDIBULARIS V

The r. mandibularis V (figs. 1 and 4, *R.Md.V*) arises from the distal and ventral end of the Gasserian ganglion slightly posterior to the origin of the r. maxillaris V. Its general course is ventral and posterior. This nerve, like the r. maxillaris, is large and easily followed at this stage. The first twig given off (fig. 1, *Mo.1*) runs dorsal and mesial to enter the primordium of the mandibular muscles. This primordium lies on the mesial side of the nerve throughout its whole course and all the motor twigs run mesially to enter it, while the sensory twigs have a lateral direction. The second twig (fig. 1, *S.1*) runs dorsally and laterally to the ectoderm and is general somatic sensory. The third twig (fig. 1, *R.Com.*) runs ventrally and slightly laterally and joins the r. maxillaris V, as mentioned above.

There can be little doubt from the character of the connection of this anastomosing branch that its fibers run from the mandibularis to the maxillaris and that, since the maxillaris supplies neither lateral line organs nor muscles, the fibers are somatic sensory and destined for the ectoderm, although they could not be followed after entering the r. maxillaris. The fourth (fig. 1, *Mo.2*) and fifth (fig. 1, *Mo.3*) twigs are motor and enter the primordium of the mandibular muscles. Their course after leaving the main nerve is ventral and mesial. The sixth twig (fig. 1, *S.2*) is sensory and runs to the ectoderm. The seventh (fig. 1, *Mo.4*) is motor and arises nearly opposite the sixth. It runs medially and enters the primordium of the mandibular muscle. The remaining four twigs (fig. 1, *S.3–6*) seem to be sensory; at least they do not enter the primordium of the muscle, since they extend beyond the distal extremity of the muscle. Neither do they enter the ectoderm but disappear near the ectoderm and, like the terminal twigs of the r. maxillaris, have the appearance of growing nerves. There cannot be much doubt that they are somatic sensory fibers.

8. THE DORSAL LATERALIS GANGLION OF VII

This large ganglion (figs. 1 and 4, *G.L.VIID.*) is triangular in form with the r. oph. sup. VII arising from its anterior angle, the r. buccalis arising from its ventral angle and the root of the ganglion representing the third somewhat truncated angle. It lies lateral to the Gasserian ganglion, which it conceals in part from the lateral view and comes into contact with the distal and ventral end of it where the r. max. V and r. mand. V. arise. There is, however, no fusion at this stage. On its posterior and dorsal border it comes into contact with the anterior end of the VIII ganglion. This point of contact consists of a rather close fusion in the specimen plotted, but in a 20 mm. embryo the line separating the two masses of cells is quite distinct and the two roots can be identified up to the point where they enter the brain wall.

The root of the dorsal lateral line ganglion of VII (fig. 1, *Rt.L.VIID.*) is massive and enters the brain as the most anterior division of the large root, of which the root of the auditory ganglion and those of the remaining ganglia of VII compose the posterior division. These relations are not so evident in the specimen plotted as in a 20 mm. embryo where there is less fusion. However they can be made out after seeing them in the younger specimen.

9. RAMUS OPHTHALMICUS SUPERFICIALIS VII

The r. oph. sup. VII (figs. 1, 2 and 3, *R.O.S.VII*) runs from the anterior angle of the dorsal lateralis VII ganglion and forms a great semicircle curving around the anterior border of the eye and terminating at a point nearly ventral to the middle of the eye and near the olfactory capsule. Its position is always quite near the ectoderm. It is a pure lateral line nerve and supplies fibers to two large primordia of lateral line organs (fig. 1, *L.1* and *L.2*). The first of these lies dorsal to the eye and the nerve gives off three well defined twigs, the most posterior of which divides as it enters the primordium. This primordium evidently represents the most posterior organs of the supraorbital

line. The second primordium lies ventral to the anterior border of the eye and just anterior to the nasal capsule. There are six or seven twigs given off to this primordium which represents the anterior supraorbital lateral line organs.

10. RAMUS BUCCALIS VII

The ramus buccalis VII (figs. 1 and 4, *R.B.VII*), a pure lateral line nerve, arises from the ventral angle of the dorsal lateralis VII ganglion. It pursues a course directly ventral and slightly posterior to that of the r. max. VII, which it conceals partially from the lateral view. At the point of exit of this ramus from the ganglion and in the angle formed by the r. buccalis and r. oph. sup. VII on the anterior border of the ganglion arise two short twigs which run laterally to a primordium of a lateral line organ (fig. 1, *L.1*). This is apparently the primordium of the most posterior organs of the infraorbital line. When the r. buccalis reaches the level of the ventral border of the lens it gives off a twig to a primordium of lateral line organs (fig. 1, *L.3*), after which it runs slightly cephalad and ventral to end under the posterior border of the eye. Near its termination it gives off a twig to a second primordium of lateral line organs (fig. 1, *L.4*). Beyond this point it becomes an extremely delicate twig and disappears while in contact with the ectoderm. While there are no primordia of lateral line organs beyond this point, the relation of the terminal ramus to the ectoderm leaves no doubt that it is lateralis in type.

At the opposite posterior border of the ganglion and at a slightly more dorsal level near the point of contact between the dorsal lateralis ganglion with the auditory ganglion arises a second twig (fig. 1, *R.O.*), the first three divisions of which innervate a lateral line primordium (fig. 1, *L.2*) which is located where the supraorbital and infraorbital lines will probably join. This cannot be stated definitely, of course, since the lateral line organ primordia are discontinuous at this stage, as in Ameiurus (Landacre, '10). It would be interesting to follow the history of these primordia in a close series in view of the author's hypothesis based on a study of Lepidosteus (Landacre and Conger,

'13) that lateral line primordia arise from discontinuous areas
rather than from a continuous area on the ectoderm. After
supplying three twigs to the lateral line primordium mentioned
above, the ramus continues beyond this point and disappears
near the anterior border of the spiracular gill cleft. The nature
of this terminal twig could not be determined. This nerve is
identified provisionally as the r. oticus VII.

11. THE ACUSTICO-FACIALIS GANGLIONIC COMPLEX

These ganglia are rather closely fused, especially at their
proximal ends, in the specimen plotted, but with the aid of a
20 mm. embryo the relations seem to be intelligible. The genic-
ulate (fig. 1, *G.Gen.*) and ventral lateralis VII (fig. 1, *G.L.VII
V*) form the ventral portion of a V-shaped mass, of which the
auditory ganglion (fig. 1, *G.Au.*) forms the dorsal arm. The
apex of the V projects cephalad and is formed by the point of
union of these two masses near their roots. The apex of the V
is in contact with the dorsal lateralis VII ganglion on its posterior
surface. The ventral arm of the V extends caudad and slightly
ventral, while the dorsal arm formed by the auditory is approxi-
mately horizontal. The most anterior member of the group is
the geniculate, which lies on the ventral and anterior border
of the ventral arm of the V. The ventral lateralis VII lies
slightly dorsal and lateral to the geniculate partly concealing the
geniculate from a lateral view. The VIII ganglion is partly
concealed by the auditory vesicle.

The root of this complex enters the brain along with that of
the dorsal lateralis VII ganglion and occupies a position posterior
and mesial to the root of that ganglion. Reading from posterior
to anterior the first root encountered, that of the auditory (fig.
1, *Rt.Aud.*), lies lateral to the two succeeding roots and enters
in conjunction with that of dorsal lateralis VII. The next root
(fig. 1, *Rt.Gen.*) encountered is that of the geniculate accom-
panied by the motor fibers of the r. hyomandibularis. The third
root (fig. 1, *Rt.L.VII V*) encountered is that of the ventral
lateralis VII. These last two roots mentioned leave the proxi-
mal end of the combined ventral lateralis VII and geniculate

ganglia in the reverse order, i.e., the root of the ventral lateralis is lateral and somewhat posterior in position and in their course from the ganglion to the brain wall they cross so that the root of the ventral lateralis ganglion enters more anterior than that of the geniculate and motor portion of the r. hyomandibularis. The sensory and motor fibers of the combined geniculate and motor root could not be followed separately, since they form a compact bundle.

12. THE AUDITORY GANGLION AND RAMI

The auditory ganglion (figs. 1 and 5, *G.Au.*) will be treated first, since its relations are much simpler than those of the remainder of the complex. The ganglion is well isolated throughout most of its extent, especially in the 20 mm. stage, and its ventral and anterior boundary, which is the one in contact with the remainder of the complex, can be recognized up to the point where it comes into contact with the posterior border of the dorsal lateralis VII; from this point the two masses of entering fibers are distinct but their accompanying cells cannot be distinguished. The most anterior ramus arising from the auditory ganglion, enters the auditory vesicle on its ventral and lateral border, while the more posterior rami enter the vesicle on the posterior and mesial border. The undeveloped condition of the auditory vesicle makes it difficult to identify these rami definitely, since the sensory areas of the auditory vesicle are not differentiated. The more anterior ramus seems to be connected with the saccular portion and the two posterior rami with the utricular portion.

13. THE GENICULATE GANGLION

The geniculate ganglion (figs. 1 and 5, *G.Gen.*), the most anterior ganglion of the VII and VIII complex, as mentioned above under the general discussion of the VII and VIII complex, lies ventral and slightly mesial to the ventral lateralis VII. The ganglion can be identified throughout its whole extent, although it is in contact with the ventral lateralis VII. At the

point of exit of the ramus hyomandibularis, however, the fibers from the geniculate and from the ventral lateralis VII fuse into a compact trunk in which the different components cannot be identified. The roots of these two ganglia, as mentioned above can be distinguished.

In form the geniculate ganglion is roughly triangular with the root representing the dorsal angle, the origin of the ramus palatinus and the ramus pretrematicus representing the ventral angle and the r. hyomandibularis representing the posterior angle. In the specimen plotted no distinction could be made out between general visceral cells and special visceral cells derived from the epibranchial placode. In a 20 mm. embryo, however, Reed ('16) was able to identify these cells and there is, further, in the specimen plotted a slight contact between the geniculate ganglion and the ectoderm at the point at which the placode proliferated cells which were added to the ganglion.

14. RAMUS PALATINUS AND RAMUS PRETREMATICUS

These two nerves arise from the ventral angle of the geniculate ganglion where it rests on the anterior face of the spiracular gill cleft. Just at the point of emergence of the larger r. palatinus, a small twig, the ramus pretrematicus (fig. 1, *R.Pr.VII*) or ramus prespiracularis VII, arises and immediately divides running caudad along the anterior wall of the spiracular cleft. Beyond this point the r. palatinus (figs. 1 and 5, *R.Pal.VII*) passes nearly ventral in direction, dividing into two twigs neither of which reaches the endoderm of the pharynx. Both, however, pass in a mesial and ventral direction and come into close relation with the endoderm. While several of the finer divisions of these twigs can be identified last in the loose mesenchyme near the pharyngeal endoderm, there is every reason, from the behavior of these nerves in other types and the absence of muscle primordia in their vicinity, for identifying them all as visceral sensory nerves.

At the posterior angle of the ganglion there is given off, at the point where the geniculate ganglion joins the ventral lateral line ganglion, a ramus which immediately fuses so closely with

fibers from the lateral line ganglion forming the ramus hyomandibularis that the two components are indistinguishable. Consequently the ramus hyomandibularis will be described separately, since it contains not only visceral sensory fibers from the geniculate but lateral line fibers and motor fibers as well.

15. THE VENTRAL LATERALIS VII GANGLION

The ganglion identified as ventral lateralis VII (figs. 1 and 5, *G.L.VII V.*) occupies the position with reference to the geniculate and auditory usually held in the embryos of fishes and amphibians (Landacre, '14) but is surprisingly large in proportion to the single lateral line primordium innervated by the r. hyomandibularis into which all the fibers from this ganglion pass. This disproportion may be explained on the basis that, while the r. hyomandibularis innervates in the adult at least five lateral line organs in the hyomandibular line, only one has appeared at this stage. The ventral lateralis VII is approximately as large as the geniculate and rather closely fused with it, although, as stated in discussing the geniculate, it can at all points in the contact be distinguished from it except in the r. hyomandibularis, and further the root of the lateral line ganglion can be traced into the brain where it enters with that of the dorsal lateralis VII. The ventral lateralis ganglion is crescent-shaped with the convexity on the ventral side and no pure lateral line rami leave it at this time so that the evidence for its identity except as presented above is not so definite as for the other members of the acustico-facial complex of ganglia.

16. TRUNCUS HYOMANDIBULARIS

The truncus hyomandibularis (fig. 1, *R.Hyo.VII*) is a mixed nerve quite compact in structure and easy to follow but somewhat difficult to analyze. It arises from the posterior fused ends of the geniculate and ventral lateralis VII. It pursues a course slightly ventro-caudad to a point where it gives off a motor twig (fig. 1, *Mo.1*) to the primordium of the hyoid musculature, then turns directly ventral. It gives off next three twigs

(figs. 1, *S.1, 2* and *3*) which run toward but do not reach the endoderm. They are certainly not motor and are apparently visceral sensory. Beyond this point there is given off a twig which runs slightly cephalad and dorsal to end on a primordium of a lateral line organ (fig. 1, *L.1*). Opposite the lateral line twig is given off a long motor twig (fig. 1, *Mo.2*) which runs ventral and caudad and after giving off several motor twigs enters the extreme end of the primordium of the hyoid muscles. Between the lateral line and the motor twigs arise two large twigs (figs. 1, *S.4* and *5*) which run directly to the ectoderm.

The ectoderm at this point is slightly thickened but not sufficiently differentiated to enable one to determine positively whether the thickening is that of a lateral line primordium or of gustatory organs. It has more the appearance of early gustatory organs and I have identified these twigs as visceral sensory, although so far as their appearance and mode of termination is concerned, aside from the slight thickening of the ectoderm, they might be general somatic sensory. The evidence against this view rests on the absence of any recognizable somatic sensory ganglion on this nerve at this time and the absence of any connecting ramus from the Gasserian ganglion. This is said to be present in the adult.

In view of the fact that there are said to be not only general somatic fibers in the VII which may come from the Gasserian ganglion but that in certain types such as Amblystoma (Landacre, '14, note on p. 603) there are fibers of this character in the VII and, further, that Norris ('13) has described a general cutaneous ganglion on the VII, a careful search was made in the type plotted for such a ganglion, especially in view of the difficulty of determining the character of the fibers mentioned above. No isolated ganglionic mass aside from those already described could be identified either on the 22 mm. or on older specimens. However, the late differentiation of the general cutaneous ganglia and the small size of their cells, making them hard to distinguish from the indifferent cells found on the roots of all nerves, render it unsafe to say that there are no such cells or fibers in the VII nerve. This interesting point must be

settled on older material than that at my disposal. From the material at hand the evidence seems to be against such a view. If they are found in other vertebrate types they should certainly be expected in such a generalized type as the shark.

17. THE GLOSSOPHARYNGEAL GANGLION AND ROOT

The glossopharyngeal ganglion is elongated in its dorso-ventral axis, extending from the middle of the medulla ventrally and slightly caudad nearly to the level of the roof of the pharynx. It contains two easily recognizable divisions; the proximal is the lateralis IX ganglion (figs. 1 and 6, *G.L.IX*) and the distal and ventral division is the visceral division or ganglion petrosum (figs. 1 and 6, *G.V.IX*). The proximal division extends from the point of contact with the medulla to the point of origin of the ramus supratemporalis IX (figs. 1 and 6, *R.St.IX*). The two ganglionic masses are in contact at this point and cannot be distinguished with certainty but throughout the remainder of the extent of the lateralis ganglion the visceral ganglion is represented by a fibrous root apparently not accompanied by ganglion cells. A short distance ventral to the origin of the ramus supratemporalis the lateral line ganglion cells cease and it could not be determined with certainty that no lateral line fibers entered the truncus glossopharyngeus. No lateral line primordia are innervated, however, by that nerve beyond those mentioned below and presumably no lateral line fibers enter it at this stage.

The root of the lateral line IX (fig. 1, *Rt.L.IX+X*) passes dorsally, mesial to the posterior end of the auditory capsule, along with visceral sensory and motor fibers of the truncus glossopharyngeus. In that part of their course between the proximal end of the ganglion and the medulla both the lateral line root and the visceral sensory and motor roots are fibrous and form a compact bundle. Unless, however, the lateral line fibers change their position in this region of the root, the visceral fibers, both motor and sensory, enter at a somewhat more ventral level where they join a more mesial column than the

lateral line fibers. The lateral line fibers join those of the lateral line root of X and enter at a somewhat more dorsal level, passing into a well defined column in contact with the limiting membrane of the lateral wall of the medulla.

The ganglion petrosum or visceral IX (figs. 1 and 6, *G.V.IX*), as mentioned above, begins at the point of origin of the ramus supratemporalis IX and extends ventrally and caudally to the dorsal border of the gill pocket. Its distal end is still in contact with the epibranchial placode (fig. 7, *G.V.IX+Pl*) and cells are evidently being added to the ganglion in the specimen plotted. From the distal end of the ganglion extends caudally a large mass of cells (fig. 1, *G.P.IX*) closely in contact with the ectoderm, which is apparently not yet fully incorporated into the ganglion giving it a curious form. The same appearance is presented by the visceral portion of the VII ganglion in a 26 mm. embryo. This mass will probably be incorporated with the remaining cells to give the slender spindle-shaped ganglion of the adult. Throughout the whole extent of the petrosal ganglion the visceral motor component of the truncus glossopharyngeus can be followed, but in the root of the ganglion, motor and sensory fibers are so closely fused that they cannot be separated. They enter the medulla somewhat more ventral than the lateral line root but at the same anterior-posterior level (fig. 1, *Rt.Vis.IX*).

18. RAMUS SUPRATEMPORALIS IX

The ramus supratemporalis IX (figs. 1 and 6, *R.St.IX*) arises from the distal end of the lateralis IX ganglion, from which point it runs directly lateral then curves slightly posterior and then runs dorsal and slightly anterior. The first twig is given off a short distance from its exit from the ganglion and ends on a small primordium of a lateral line organ (fig. 1, *L.1*). A second small twig (not named on figure 1), arises at the same point runs slightly more dorsal and comes quite close to the ectoderm but does not enter it. The ectoderm is not modified at this point and the nature of this twig could not be identified. It re-

sembles a general cutaneous nerve but the absence of any iso-
lated general somatic ganglion argues against this view. It is
more probable that it is a special visceral sensory nerve such as
accompanies the ramus supratemporal IX in Menidia (Herrick,
'99). The visceral sensory ganglion on IX is so situated that
fibers from that ganglion could readily enter the ramus supra-
temporalis. From the point of origin of these two twigs the
ramus supratemporalis curves dorsal and cephalad to end on the
primordium of a lateral line organ (fig. 1, *L.2*) situated almost
directly lateral to the proximal end of the ductus endolymphat-
icus.

19. RAMUS PHARYNGEUS AND RAMUS PRETREMATICUS IX

These two rami arise together as one nerve from the middle
of the anterior border of the ganglion petrosum. The first
twigs to be given off are the pretrematic rami (figs. 1 and 6,
R.Pr.IX) which curve caudad and end on the epithelium of the
gill bar. The second ramus or ramus pharyngeus (figs. 1 and 6,
R.Ph.IX) turns ventral and mesial and after pursuing a much
longer course comes into direct contact with the endoderm of the
roof of the pharynx. All these rami are evidently visceral
sensory.

20. TRUNCUS GLOSSOPHARYNGEUS

The truncus glossopharyngeus (figs. 1 and 7, *R.PO.IX*) arises
from the distal end of the ganglion petrosum and is a combined
sensory and motor root containing visceral sensory and visceral
motor fibers so closely combined that they cannot be distin-
guished. This nerve runs ventral and slightly caudad to the
level of the floor of the pharynx. The first twig given off is
sensory, arises quite close to the ganglion and runs to the endo-
derm of the gill bar. The third twig (fig. 1, *Mo.1*) is motor
entering the primordium of the branchial musculature as do all
the motor twigs of this nerve. The second, fourth and fifth
(figs. 1, *S.2, 3* and *4*) are sensory and run to the epithelium of the
gill bar. The sixth twig (fig. 1, *Mo.2*) seems to be motor, as
does also the seventh and terminal twig. However, the muscle

primordia are at this time poorly developed and one or more
of these twigs may be visceral sensory. No lateral line organ
primordia are present at any point innervated by the truncus
glossopharyngeus and all its sensory fibers seem to be visceral
sensory.

21. THE VAGUS GANGLIA

The vagus ganglion is irregular in form. It extends in the
longitudinal axis from the point of entrance of the IX caudad to
the level of the third spinal ganglion. Its dorsal portion is thin
from mesial to lateral but is continuous from the point of en-
trance of the IX to a point directly over the dorsal border of the
second true gill slit, from which point it extends caudally as a
narrow strand of cells which is continuous with the first spinal
ganglion. This proximal portion of the ganglion, which con-
tains root fibers and the primordium of the somatic sensory or
jugular X ganglion, is continued ventrally by five branchial
ganglia. The anterior branchial ganglia are well isolated but the
posterior ones are somewhat more fused. The proximal portion
of each of the first three branchial ganglia is chiefly lateral line
plus root fibers and will be designated as lateralis ganglia X_1,
X_2, X_3. The distal portion of all five is visceral sensory and
will be designated as visceral ganglia X_1, X_2, X_3, X_4, X_5.

The lateral line rami arise from the dorsal and lateral borders
of the proximal or lateral line portions, while from the distal or
visceral portions arise the pretrematic, posttrematic and motor
rami. All the branchial ganglia extend from their proximal
ends in a ventral and caudal direction and all the visceral sen-
sory ganglia of X are still in contact with their respective epi-
branchial placodes and are still receiving cells from these sources
as in the 20 mm. embryo (Reed '16). The proximal portion of
the first two branchial ganglia (including the lateralis ganglion
X_1 and the jugular ganglion associated with branchial X_1 and
X_2) and nearly all of the lateralis ganglion X_2 (including of
course all roots of X) lie mesial to the primordium of the somatic
musculature. The distal portions of visceral X_1 and X_2 and
X_3 lie lateral to the muscle primordium while the lateral line
ganglion X_3 and the remainder of visceral X_4 and X_5 lie ven-

tral to this muscle primordium. The epibranchial portions of X_4 and X_5 which are still attached to the ectoderm are more lateral in position and not directly under the muscle primordium. The muscle primordium is pierced by the proximal end of the visceral ganglion of branchial X_2.

22. THE VAGUS ROOTS

The analysis of the vagus roots in detail beyond the number and point of attachment is very difficult and sometimes impossible in a 22 mm. embryo.

The first root of X (fig. 1, $Rt.L.IX+X$) is a lateral line root and enters along with the lateral line root of IX just dorsal to the visceral sensory and motor roots of IX. Posterior to this most anterior root are three roots much alike in appearance. These three roots arise anterior to the level of the point of origin of the r. supratemporalis X. The second, third and fourth roots arise from the thicker anterior portion of the X ganglion, while the first or lateralis root, joins the brain wall only after a rather long course cephalad as is usually the case with the lateral line root of X which connects the X ganglion with the IX.

Each of the second, third and fourth roots is round in transverse section, contains a rather large number of cells on the anterior face of the root, and as it enters the brain wall, divides into two divisions one of which turns slightly dorsally and the other ventrally and mesially. Owing to the very minute size of the general cutaneous rami of X the dorsal division is identified provisionally as visceral sensory and the more ventral division as visceral motor. The more dorsal fibers do not enter the tract which the lateral line fibers of IX and X from the first root enter, but do enter the same column entered by the more ventral or visceral root of IX. Caudally of the first four roots there are twelve to fifteen roots (not named on figure 1) arising from the more attenuated caudal portion of the ganglion, all of which show the same composition as the second, third and fourth. They are slightly smaller and each root on entering the medulla divides into a dorsal and a ventral branch. The dorsal division becomes progressively smaller in the more posterior roots, and

some of the posterior roots on the opposite side from that plotted, are made up exclusively of the ventrally directed roots. These roots are identified provisionally, for reasons given above, the dorsal as visceral sensory, and the ventral as visceral motor.

23. THE JUGULAR XTH GANGLION

The mass of cells identified as the jugular or general cutaneous ganglion of X (figs. 1 and 7, $G.J.X.$) is situated in the proximal portion of the X complex. It lies lateral to the proximal attenuated fibrous root and extends from the level of the anterior end of the lateralis ganglion on branchial X_1, to the middle of the lateralis ganglion on X_2. It lies dorsal to both these ganglia where it comes into contact with them and, except for the fibrous bundle running between X_2 and X_1, it forms the ventral boundary of the Xth complex in this region. This ganglion is composed of small cells and is apparently in a very immature condition, as is the same ganglion in Ameiurus and Lepidosteus (Landacre, '10 and '12) in approximately the same stage of development. No root fibers from this ganglion could be identified.

The mass of cells described above as the jugular ganglion is found in a 20 mm. embryo, but is better defined in a 25 mm. embryo, where it is well isolated from the ganglia lateralis X_1 and X_2 with which it is in contact on its posterior and ventral surface. In a 30 mm. embryo this mass of cells is not isolated but seems to be fused with lateralis X_3. If this interpretation is correct, it has migrated, between the 25 mm. stage and the 30 mm. stage, from a position mesial to the somatic muscle primordium to a position lateral to this primordium. This is equivalent to a migration from an intracranial to an extracranial position.

24. RAMUS AURICULARIS

The ramus auricularis (figs. 1, 9, 10 and 11, $R.Aur.$) or ramus cutaneous dorsalis vagi has not been identified with certainty. There are certainly no well defined nerves of this character arising from the dorsal and proximal portion of the Xth gan-

glionic complex which is the usual place of origin of this nerve in embryos. All the embryos at my disposal from 20 mm. to 30 mm. have been examined repeatedly with the greatest care and no nerves pass dorsally to the ectoderm from the proximal portion of the ganglion. This is true of the 25 mm. embryo, where the mass of cells identified above as the jugular ganglion is best isolated.

There are, however, in the 22 mm. and 25 mm. embryos several minute processes arising from the posterior and dorsal border of the root of X which could not be followed farther than the width of one ganglion cell but present the appearance of very immature nerves. They are constant in neither number nor position and vary in both on opposite side of the same embryo and are not included in the reconstruction in figure 1.

In the 30 mm. embryo a nerve arises apparently from the root of X near the first spinal ganglion but not from it and runs cephalad passing along the ventral border of the anterior end of the primordium of the somatic musculature then runs to the ectoderm of the mid-dorsal region of the head where it ends just posterior to the area supplied by the most dorsal rami of supratemporalis IX and X. This nerve is very small and could not be located on the opposite side of the same specimen. It has the usual distribution of a r. auricularis in embryos except that it arises too far posterior.

There is in addition in all embryos from 22 mm. to 30 mm. in length a nerve (figs. 1, 9, 10 and 11, $R.Aur.$) running out with the lateral line ramus arising from the ganglion lateralis X_2. It arises with the lateral line nerve but soon separates from it and pursues a course caudad parallel to it but slightly more ventral in position than the lateral line nerve to a point near the third spinal ganglion, when it turns dorsal and is distributed to the ectoderm. Its relation to the lateral line nerve is not entirely clear since it seems to form anastomoses with it but some of the fibers of this nerve are distributed to the ectoderm at points where there are no lateral line primordia at the stages studied.

The fibers seem to pursue a course from the distal end of the jugular ganglion proximally along the dorsal border of lateralis

X_3. This nerve is identified provisionally as the ramus auricularis. Both the jugular ganglion and its nerve are surprisingly small and there seems to be a large amount of ectoderm on the posterior portion of the head devoid of general cutaneous innervation at the stages studied. Both the nerves identified as supratemporal rami were carefully examined for general cutaneous fibers without success. The morphology of the r. auricularis X has been treated fully by Herrick ('99, p. 267–273). A more detailed description of this nerve requires older material than that at my disposal.

25. THE GANGLION LATERALE X_1

There are three lateral line ganglia on the Xth nerve occupying the proximal portions of the first four branchial ganglia which will be designated in the description as Laterale X_1, X_2 and X_3 respectively. The most anterior or laterale X_1 (figs. 1 and 7, $G.L.X_1$) is situated on the proximal portion of branchial X_1. It extends from the anterior end of the jugular ganglion ventrally and caudally along the root fibers of branchial X_1 almost to the proximal end of the ganglion viscerale X_1. It does not at this stage come into contact with that ganglion, there being a short fibrous root of viscerale X_1 containing no cells. On its proximal end it is in contact with the jugular ganglion to which it is ventral in position.

Throughout the whole length of the lateralis X_1 ganglion the root of the ganglion viscerale X_1 and motor X lie mesial to it. This ganglion is compact and nearly round in transverse section except at the origin of two lateral line rami, where a large mass of cells projects laterally toward the ectoderm making the ganglion triangular in form. Its cells are large and readily distinguished from those of the jugular X, with which it is in contact dorsally. The cells of this lateral line ganglion are not readily distinguished from those of the visceral ganglion, but this produces no confusion here since these two ganglia are not in contact.

26. RAMUS SUPRATEMPORALIS VAGI

The ramus supratemporalis X (figs. 1 and 7, *R.St.X*) arises near the middle of the lateral surface of the ganglion laterale X_1 from a prominent mass of cells that extends from the ganglion nearly to the ectoderm. Immediately after its origin the ramus divides into two twigs, posterior and anterior, the posterior nerve running nearly directly caudad and the anterior larger twig curving dorsal and cephalad. The anterior twig innervates the primordia of two lateral line organs, of which the proximal one (fig. 1, *L.2*) lies directly over the point of origin of the combined ramus and at the dorso-ventral level of the attachment of the roots of X to the medulla. The other organ (fig. 1, *L.1*) lies farther dorsal and anterior at the level of the entrance of the second root of X and at a dorso-ventral level of the dorsal border of the auditory vesicle. The posterior twig supplies a long primordium of lateral line organs (fig. 1, *L.3*), to which it gives off two twigs, indicating that there will be at least two organs derived from this primordium in the adult. This lateral line primordium lies directly over the visceral ganglion of branchialis X_1 and just ventral to the level of the posterior roots of X. It is placed diagonally to the long axis of the body with the anterior end more dorsal.

In a 20 mm. embryo this primordium and the more posterior of the two innervated by the anterior twig are continuous, while the most anterior lateral line primordium is not present. The rapid appearance of these primordia at this time renders difficult the exact identification of the organs as belonging to the main head lateral line or as being accessory. The occipital lateral line commissure is not yet formed and these organs, including the one innervated by supratemporalis IX, are identified as the last four organs of the head posterior to the junction of infraorbital and supraorbital lines, the primordium innervated by the most proximal and posterior twig of dorsal lateralis VII being considered as the point of future junction of supraorbital and infraorbital lines. The primordium innervated by

the posterior twig of lateralis X_1 may belong to the main body line. It lies much more ventral in position than those innervated by the anterior twig.

27. THE GANGLION LATERALE X_2

The lateralis ganglion (figs. 1 and 8, $G.L.X_2$) associated with the second branchial ganglion of X resembles that on the first branchial ganglion of X in its relations to other members of the complex. It is, however, longer and placed parallel to the long axis of the body. It is not so definitely confined to its branchial ganglion as that on X_1, since its posterior end is continuous with lateralis X_3. Its proximal and anterior end is mesial to the somatic muscle primordium and is in contact with jugular X, to which it lies ventral. Throughout its whole course it lies lateral to the fibrous motor and sensory root of the remaining visceral ganglia of X. Its posterior and distal end lies lateral to the somatic muscle primordium. On its extreme distal and posterior end its cells are continuous with those of lateralis X_3 and throughout the posterior half of its extent it is in contact with the visceral ganglion of branchial X_2, to which it lies dorsal. Throughout the proximal portion of this contact the two ganglia are closely fused and, owing to the similarity in size of their cells, indistinguishable. However, throughout the greater portion of the contact the combined ganglia are indented both on the mesial and on their lateral surfaces, indicating the line of separation between them. Near its posterior end this ganglion gives rise to the first ramus lateralis X.

28. FIRST RAMUS LATERALIS X

Owing to the position of the lateral line primordium innervated by the lateral line ramus arising from this ganglion, it is identified as the first primordium of the main body line and its nerve as the first lateral line ramus of X, rather than as a homologue of the more anterior ramus supratemporalis X. The study of this nerve in much older material, however, may show it to be homologous to a supratemporal ramus.

This nerve (figs. 1, 9, 10 and 11, *R.L.X.1*) arises near the posterior end of ganglion laterale X_2 accompanied by the nerve identified as ramus auricularis. Immediately after its exit from the ganglion it gives off a small twig to the lateral line primordium. Posterior to this twig at least two more twigs are given off to the same primordium which extends caudad to the level of the first spinal ganglion. Posterior to this point the terminal twig of this nerve can be followed, but it does not end on a lateral line primordium. There is nothing in its relation to the ectoderm, other than the absence of lateral line primordia posterior to this point, by which to identify it. The extremely immature condition of the general cutaneous rami of X make it difficult to be certain of its identity. The only other possibility apparently is that it might be a visceral sensory twig destined for terminal buds on the ectoderm. This can be determined, however, only on older material and it is indicated on the plot as a lateral line nerve.

29 THE GANGLION LATERALE X_3

This ganglion (figs. 1, 9, 10 and 11, $G.L.X_3$) shows many of the characteristics of lateralis X_2. It is longer and placed slightly more diagonally in the body with its anterior end more dorsal than its posterior end. The ganglion is round in transverse section and lies throughout its whole extent lateral to the primordium of the somatic musculature.

At its anterior and proximal end it is in contact and continuous with lateralis X_2 on its dorsal surface, while on its ventral surface it is contact for a short distance with the visceral ganglion of X_2. The remainder of its dorsal surface is free, but on its ventral surface it is in contact first with the visceral ganglion of X_3, and at its posterior end for a short distance with the visceral ganglion of X_4. Its ganglion cells are much larger than the visceral ganglion cells and their identification is easy. The fibrous motor and sensory roots of the complex posterior to this point lie on the ventral portion of lateralis X_3 instead of on the mesial surface, as in the case of the two preceding lateral line ganglia.

30. SECOND AND THIRD RAMI LATERALES X

The lateral line trunk (figs. 1, 12 and 13, $R.L.X.2$) for the body lateral line organs arises by two rami from the dorsal and lateral surface, near the posterior end of lateralis X_3, the ganglion cells continuing caudad for a short distance beyond the second of the two twigs which make up this ramus. They arise near together and on the side plotted remain distinct but on the opposite side of the same embryo after a short course as separate twigs combine into a single ramus which retains nearly its original size back to the level of the middle of the yolk stalk, where my series ends.

From the anterior twig several small branches run to thickenings of the skin which were identified as primordia of lateral line organs.

31. THE GANGLIA VISCERALIA X_1 TO X_4

The visceral sensory portions of the branchial ganglia of X (fig. 1, $G.V.X_2$ to X_5; figs. 8 to 12, $G.V.X_1$ to $X_5 + Pl$) are all similar in form with the exception that the first three are much better isolated than the remaining two. All give rise to pharyngeal and pretrematic and posttrematic rami. All these visceral ganglia are still attached to the epibranchial placodes of their respective gills and all are still receiving cells from the ectoderm. The attachment of the last three is much more intimate and more extensive in proportion to the size of the ganglion than that of the first two. The last two ganglia are much more poorly defined with more uneven borders than the anterior ones which are sharply isolated from the surrounding mesenchyme. The first three ganglia are spindle-shaped, round in transverse section, placed diagonally in the body with the proximal end slightly anterior, and end distally in the enlargement formed by their fusion with their epibranchial placodes. The posterior extremities of the last two are similarly attached, but these two ganglia are fused in their proximal portions. From the point of attachment of each ganglion to its placode there is a large cellular mass which extends caudad from the main body of the ganglion. This mass presumably will be

incorporated into the main body of the ganglion, as in the visceral ganglion of VII. There are at this stage five of the true gill slits open, but there is posterior to the last gill slit an epibranchial ganglion. This is interpreted as a vestigial fifth branchial ganglion of X.

32. FIRST TRUNCUS BRANCHIALIS X

There are three rami arising from the distal and ventral end of the first ganglion branchialis vagi. The first nerve is the ramus pharyngeus (fig. 1, $R.Ph.X_1$) which runs mesially and ventrally to the roof of the pharynx. The second ramus is the ramus pretrematicus (figs. 1 and 8, $R.Pr.X_1$) which is sensory and comes into contact with the anterior border of the gill slit. Both these rami arise from the ganglion where it is still in contact with the epibranchial placode and will doubtless contain gustatory fibers.

The posttrematic nerve (figs. 1 and 9, $R.Po.X_1$) is large and arises from the extreme distal end of the ganglion. It pursues a course ventral and slightly caudal in the gill bar. The first two rami (fig. 1, *Mo.1* and *Mo.2*) given off are motor, the third (fig. 1, *S.1*) sensory, and the fourth and fifth or terminal are again motor (fig. 1, *Mo.3* and *Mo.4*). No posttrematic sensory ramus similar to that on IX could be detected.

33. SECOND TRUNCUS BRANCHIALIS X

The second branchial trunk is quite like the first, possessing a ramus pharyngeus (figs. 1, $R.Ph.X_2$) and ramus pretrematicus (fig. 1, $R.Pr.X_2$), both sensory and arising from that portion of the visceral ganglion fused to the epibranchial placode. The posttrematic nerve (figs. 1 and 10, $R.Po.X_2$) arises from the distal and ventral end of the ganglion and runs ventrally from this point. The first twig given off is motor, the second sensory, while the third and terminal twig is motor. All sensory twigs from the first and second pretrematic nerves turn laterally toward the ectoderm, while the motor twigs turn mesially to the primordium of the branchial musculature.

34. THIRD TRUNCUS BRANCHIALIS X

The third branchialis ramus repeats the pattern of the second, having a ramus pharyngeus (fig. 1, $R.Ph.X_3$) and ramus pretrematicus (figs. 1, 11, $R.Pr.X_3$) with the same relation to the epibranchial ganglion. The posttrematic ramus (figs. 1, 12, $R.PoX_3$) seems to lack sensory fibers; at least none could be detected. The terminal ramus of the third posttrematic nerve curves forward to end on the primordium of the branchial musculature, and, in fact, the whole ramus forms a gentle curve cephalad. The rami of the fourth branchial nerve are small, particularly the ramus pharyngeus and the ramus pretrematicus.

35. FOURTH TRUNCUS BRANCHIALIS X

The first division of the fourth branchial nerve arises as two minute twigs in the position occupied by the ramus pharyngeus and ramus pretrematicus on the more anterior branchial ganglia. They (fig. 1, $R.Ph.X_4$ and $R.Pr.X_4$) are identified as these nerves although their minute size prevents their being followed to their terminations. The ramus posttrematicus (figs. 1, 13, $R.PoX_4$) is much easier to follow and pursues a course behind the last gill slit ventrally, then curves slightly forward to pass to the heart, where it can be identified last near the wall of the pericardium. It is identified provisionally as the ramus cardiacus X.

36. THE GANGLION VISCERALE X_5

The fifth branchial ganglion (figs. 1, 12, 13, $G.V.X_5$) extends caudad from the fourth as a large mass of cells fully as large as any of the preceding branchial ganglia. At its posterior end it is fused with an indentation of the ectoderm, as are the more anterior branchial ganglia at their attachment to the placodes (fig. 1, $G.P.X_5$). The attachment is small and there is no corresponding pharyngeal evagination. The large bundle of fibers that has accompanied all the remaining branchial ganglia lying on their ventral or mesial surfaces, disappears in this ganglion.

37. RAMUS VISCERALIS X

There is in the specimen plotted no ramus visceralis arising from the posterior end of the ganglion visceralis X_5 such as Neal ('14, plate 7, fig. 36) plots in a 25 mm. embryo. Neither is there in my 25 mm. embryo any well defined ramus visceralis or vagus nerve, although the posterior end of this ganglion is ragged and seems to give rise to a very immature nerve trunk. This is rather surprising in view of the condition of the first two spinal nerves and the anterior sympathetic ganglia and rami communicantes, all of which are well formed in the 22 mm. embryo. There are several minute twigs arising from the ventral and mesial surfaces of this ganglion but their destination could not be determined. Their general course is mesial but they are quite short.

In a 30 mm. embryo, however, the ramus visceralis X (fig. 1, $R.Vis.\ X$) is well formed and runs directly ventrad and caudad and has the usual distribution of the vagus nerve.

SUMMARY AND DISCUSSION

Squalus acanthias possesses at the stage of 22 mm. eighteen separate cerebral ganglia. Of these eighteen ganglia the ganglia profundus, Gasserian, lateralis VII dorsalis, and acusticus, are isolated so that the nerves arising from them are pure and readily identified. The remaining ganglia are in contact with and sometimes fused with other ganglia so that, while they can be identified, they are not separate as are those mentioned above. The nerves arising from these ganglia which are in contact are mixed with motor fibers only except in the following cases. The nervus terminalis is combined with the olfactory, the ramus auricularis X seems to be fused with the ramus lateralis X_1, and the hyomandibularis contains both lateralis and visceral sensory fibers. The following table gives schematically the ganglia and rami as identified.

The nervus terminalis is placed provisionally under the general cutaneous component where it is classified by Johnston ('06, p. 106). Brookover ('10), however, presents strong evidence for

TABLE I

Showing the ganglia and rami of a 22 mm. embryo of Squalus acanthias

COMPONENT GANGLIA	LATERAL LINE NERVES	GENERAL CUTANEOUS NERVES	VISCERAL NERVES
No ganglion.........			Olfactory
Terminalis..........		Terminalis?	
Profundus..........		R. Oph. Prof.	
Gasserian..........		⎰ R. Oph. Sup. V ⎱ R. Max. V ⎰ R. Mand. V	
Lat. VII dors........	⎰ R. Oph. Sup. VII ⎱ R. Bucc. VII		
Lat. VII vent.......	⎰ R. Ot. VII ⎱ R. Hyom. VII		
Geniculate.........			⎰ R. Pal. VII ⎱ R. Prett. VII ⎱ R. Hyom. VII
Acusticus...........	Acusticus		
Lat. IX.............	R. Supt. IX		
Petrosum or Visceral IX..............			⎰ R. Ph. IX ⎱ R. Prett. IX ⎱ R. Postt. IX
Jugular X..........		R. Auricularis	
Lateral X₁..........	R. Supt. X		
Lateral X₂..........	R. Lat. X. 1		
Lateral X₃..........	R. Lat. X. 2		
Visceral X₁.........			⎰ R. Phar. X₁ ⎱ R. Prett. X₁ ⎱ R. Postt. X₁
Visceral X₂.........			⎰ R. Phar. X₂ ⎱ R. Prett. X₂ ⎱ R. Postt. X₂
Visceral X₃.........			⎰ R. Phar. X₃ ⎱ R. Prett. X₃ ⎱ R. Postt. X₃
Visceral X₄.........			⎰ R. Phar. X₄ ⎱ R. Prett. X₄ ⎱ R. Postt. X₄ ⎱ or R. Car.
Visceral X₅.........			R. Visceralis

classifying this nerve as a sympathetic nerve rather than as a general cutaneous nerve.

The roots of these ganglia, in sharp contrast with the nerves, are all complex and enter the brain, aside from the nervus ter-

minalis, in three chief divisions: the Gasserian and the profundus root complex, the VII–VIII root complex, and the IX–X root complex.

The most striking features of the embryonic ganglia of Squalus in comparison with Ameiurus and Lepidosteus (Landacre, '10 and '12) are, first, the presence of three distinct lateral line ganglia on X and, second, the immature condition of the general cutaneous ganglia on X and the absence of a separate ramus auricularis, this nerve being fused with the ramus lateralis X.1. The presence on IX of a general cutaneous ganglion in several vertebrate types including apparently man, and, particularly, the presence of general cutaneous ganglia and fibers in both VII and IX in Petromyzon (Johnston, '05) would lead one to expect them in Squalus. A careful search failed to demonstrate them. However, since the general cutaneous component on X matures very late and the jugular ganglion is always small and ill defined, a study of older material may show cutaneous ganglia and fibers in both VII and IX.

The special visceral or gustatory cells on VII, IX and X cannot in a 22 mm. embryo be distinguished sharply from the general visceral or branchial cells, although all of the branchial ganglia on X are still in contact with their respective epibranchial placodes. In a 20 mm. embryo (Reed, '16) the process of contribution of cells by the epibranchial placodes and their metamorphosis into ganglion cells can be observed.

The terminal buds or gustatory organs seem to be late in appearance. They are present in the 30 mm. embryo but material fixed in vom Rath is not particularly favorable for their identification and their number and position are not described in this paper. Taste buds could not be identified with certainty in the embryo plotted.

The branchial ganglia of Squalus are well isolated in comparison with Ameiurus and Lepidosteus at similar stages. All of the visceral ganglia on IX and X and most of the lateral line ganglia are in finger-like processes extending ventrally above their respective gill slits. In Ameiurus and Lepidosteus and particularly in Rana there is a large mass of cells from which the suc-

cessive branchial ganglia extend ventrally. In Squalus this mass of cells is replaced by the fibrous roots of IX and X and contains no cells except those of the jugular ganglion which are situated here up to the 25 mm. stage.

The very small size of the r. oph. sup. V in comparison with the large r. oph. prof. is worthy of note. The disappearance of the profundus nerve in higher forms is certainly not foreshadowed in Squalus. If we compare with these conditions the condition in Amphibia (Coghill, '01, '02, '06), where the ophthalmic nerve is treated as an ophthalmicus profundus, it raises an interesting question concerning the relationship of these forms with the higher vertebrates which have apparently lost both the ophthalmicus profundus ganglion and nerve. However, the ophthalmicus profundus ganglion is said to be present in the cat between the stages of 10 and 21 somites but its fate is not described (Shulte and Tilney, '15).

The usual conception of the sharks as generalized vertebrates is borne out by the condition of the ganglia and nerves with the possible exception of the general cutaneous component.

LITERATURE CITED

BROOKOVER, CHAS. 1908 Pinkus' nerve in Amia and Lepidosteus. Science, N.S., vol. 27, p. 913.

1910 The olfactory nerve and nervus terminalis and the preoptic sympathetic system in Amia. Jour. Comp. Neur., vol. 20.

BROOKOVER, CHAS. AND JACKSON, T. S. 1911 The olfactory nerve and the nervus terminalis in Ameiurus. Jour. Comp. Neur., vol. 21, no. 3.

COGHILL, G. E. 1901 The rami of the fifth nerve in Amphibia. Jour. Comp. Neur., vol. 11, no. 1.

1902 The cranial nerves of Amblystoma tigrinum. Jour. Comp. Neur., vol. 12, no. 3.

1906 The cranial nerves of Triton taeniatus. Jour. Comp. Neur., vol. 16, no. 4.

1916 Correlated anatomical and physiological studies of the growth of the nervous system of Amphibia. II. The afferent system of Amblystoma. Jour. Comp. Neur., vol. 26, no. 3.

HERRICK, C. JUDSON 1899 The cranial and first spinal nerves of Menidia. A contribution upon the nerve components of bony fishes. Jour. Comp. Neur., vol. 9, nos. 3 and 4.

JOHNSTON, J. B. 1905 The cranial nerves of Petromyzon. Morph. Jahr., Bd. 34, Heft 2.

1906 The nervous system of the vertebrates. P. Blakiston's Son and Co., Phila.

LANDACRE, F. L. 1907 On the place of origin and method of distribution of taste buds in Ameiurus. Jour. Comp. Neur., vol. 17.

1910 The origin of the cranial ganglia in Ameiurus. Jour. Comp. Neur., vol. 20.

1912 The epibranchial placodes of Lepidosteus osseus and their relation to the cerebral ganglia. Jour. Comp. Neur., vol. 22, no. 1.

1914 Embryonic cerebral ganglia and the doctrine of nerve components. Folia Neurobiologica, Band. 8, Nr. 6.

LANDACRE, F. L. AND McLELLAN, MARIE 1912 The cerebral ganglia of the embryo of Rana pipiens. Jour. Comp. Neur., vol. 22.

LANDACRE, F. L. AND CONGER, A. C. 1913 The origin of the lateral line primordia in Lepidosteus osseus. Jour. Comp. Neur., vol. 23, no. 6.

LOCY, WILLIAM A. 1899 New facts regarding the development of the olfactory nerve. Anat. Anz., Band 16, Nr. 12.

1905 On a newly recognized nerve connected with the forebrain of Selachians. Anat. Anz., Band 26, Nr. 2 and 3, p. 33–63; Nr. 4 and 5, p. 111–123.

MITROPHANOW, P. 1893 Étude embryogénique sur les Sélaciens. Arch. Zool. Exp., Sér. 3, tome 1.

NEAL, H. V. 1898 The segmentation of the nervous system in Squalus acanthias. A contribution to the morphology of the vertebrate head. Bull. Mus. Com. Zool. at Harvard, vol. 21, no. 7.

1914 The morphology of the eye-muscle nerves. Jour. Morph., vol. 25, no. 1; reprinted in Tuft's College Studies, Sci. series, vol. 3, no. 4.

Norris, H. W. 1913 The cranial nerves of Siren lacertina. Jour. Morph. vol. 24, no. 2.

Reed, Carlos J. 1916 The epibranchial placodes of Squalus acanthias. The Ohio Journal of Science, vol. 16, no. 8.

Scammon, Richard E. 1911 Normal plates of the development of Squalus acanthias. In Keibel's Normentafeln, Zwölftes Heft.

Shulte, H. von W. and Tilney, Frederick. 1915 Development of the neuraxis in the domestic cat to the stage of 21 somites. Annals of the N. Y. Acad. of Sci., vol. 24, pp. 319–346.

ABBREVIATIONS

At., Atrium
Au.Ves., Auditory vesicle
B., Base of brain
B.V., Blood vessel
B.A., Bulbus arteriosus
Dien., Diencephalon
D.End., Ductus endolymphaticus
Epiph., Epiphysis
G.Au., Auditory ganglion
G.Gass., Gasserian ganglion
G.Gen., Geniculate ganglion
G.J.X., Jugular ganglion of X
Gl. 1–6, Gill slits
G.L.VII.D., Dorsal lateral line ganglion of VII
G.L.VII.V., Ventral lateral line ganglion of VII
G.L.IX, Lateral line ganglion of IX
G.L.X₁, First lateral line ganglion of X
G.L.X₂, Second lateral line ganglion of X
G.L.X₃, Third lateral line ganglion of X
G.P.IX Epibranchial ganglion of IX
G.P.X₁–X₅, Epibranchial ganglia of X
G.Pro., Profundus ganglion
G.V.IX, Visceral ganglion of IX
G.V.X₁–X₅, Visceral or branchial ganglia of X
G.V.X₁ to X₅ + Pl., Visceral ganglia of X plus the placodal ganglia on X
G.Sp. 1–3, The first three spinal ganglia
Hyp., Hypophysis
H.C., Head cavity
L. 1–6, Lateral line primordia
M., Muscle primordia
Mo. 1–6, Motor rami of cerebral nerves
No., Notochord
N. III–IV–VI, Nerves III, IV and VI
N.Au., Auditory nerve
N.Olf., Olfactory nerve
N.Ter., Nervus terminalis
Par., Paraphysis
Ph., Pharynx

Pro. 1, 2, 3, Twigs of the profundus nerve
R.Aur., Ramus auricularis
R.Com., Ramus communicans
R.B.VII, Ramus buccalis VII
R.Hyo.VII, Ramus hyomandibularis VII
R.L.X.1, First lateral line nerve of X
R.L.X.2, Second lateral line nerve of X
R.Md.V, Ramus mandibularis V
R.Mx.V, Ramus maxillaris V
R.O., Ramus oticus
R.O.S.V, Ramus ophthalmicus superficialis V
R.O.S.VII, Ramus ophthalmicus superficialis VII
R.Pal.VII, Ramus palatinus VII
R.Ph.IX, Ramus pharyngeus IX
R.Ph.X₁–X₅, Pharyngeal rami of X
R.Po.IX, Ramus posttrematicus IX
R.Po.X₁–X₅, Posttrematic rami of X
R.Pr.IX, Ramus pretrematicus IX
R.Pr.X₁–X₅, Pretrematic rami of X
R.St.IX, Ramus supratemporalis IX
R.St.X, Ramus supratemporalis X
Rt.Aud., Root of auditory ganglion
Rt.Gass., Root of Gasserian ganglion
Rt.Gen., Root of geniculate ganglion
Rt.L.VII.D., Root of dorsal lateral line ganglion of VII
Rt.L.VII.V., Root of ventral lateral line ganglion of VII
Rt.L.IX+X, Lateralis root of IX and X
Rt.Vis.IX, Visceralis root of IX
Rt.X–2–3–4–5, Visceral sensory and motor roots of the second, third, fourth and fifth branchial ganglia of X
Rt.X–3–4–5, Visceral sensory and motor roots of the third, fourth and fifth branchial ganglia of X
Rt.4–5, Visceral sensory and motor roots of the fourth and fifth branchial ganglia of X

1   A reconstruction of the cerebral ganglia and early nerves of a 22 mm. embryo of Squalus acanthias. The embryo was fixed in vom Rath's fluid and mounted unstained. The length of all specimens was determined after fixation. The plot was made from the left side of the specimen at a magnification of 50 and reduced to 40 for publication and gives true proportions in the anterior-posterior and dorso-ventral diameters. The sections were 10 microns thick.

The ramus visceralis X has been added to the plot from a 30 mm. specimen.

The epibranchial ganglia on IX and X, which are presumably special visceral or gustatory, are indicated by vertical lines, as are the general visceral ganglia, since the exact limits of the cells contributed by the epibranchial placodes could not be determined. The posterior cone-shaped projections on IX and X are in each case in contact with the ectoderm.

The nervus terminalis in the specimen plotted contained two peripheral rami, while only one was found on other specimens.

The r. lateralis X. 1 is double on the side plotted but single on the opposite side of the same specimen.

The motor twigs are indicated at their separation from the chief ramus only, the whole course of the motor fibers not being shown in order to simplify the plot.
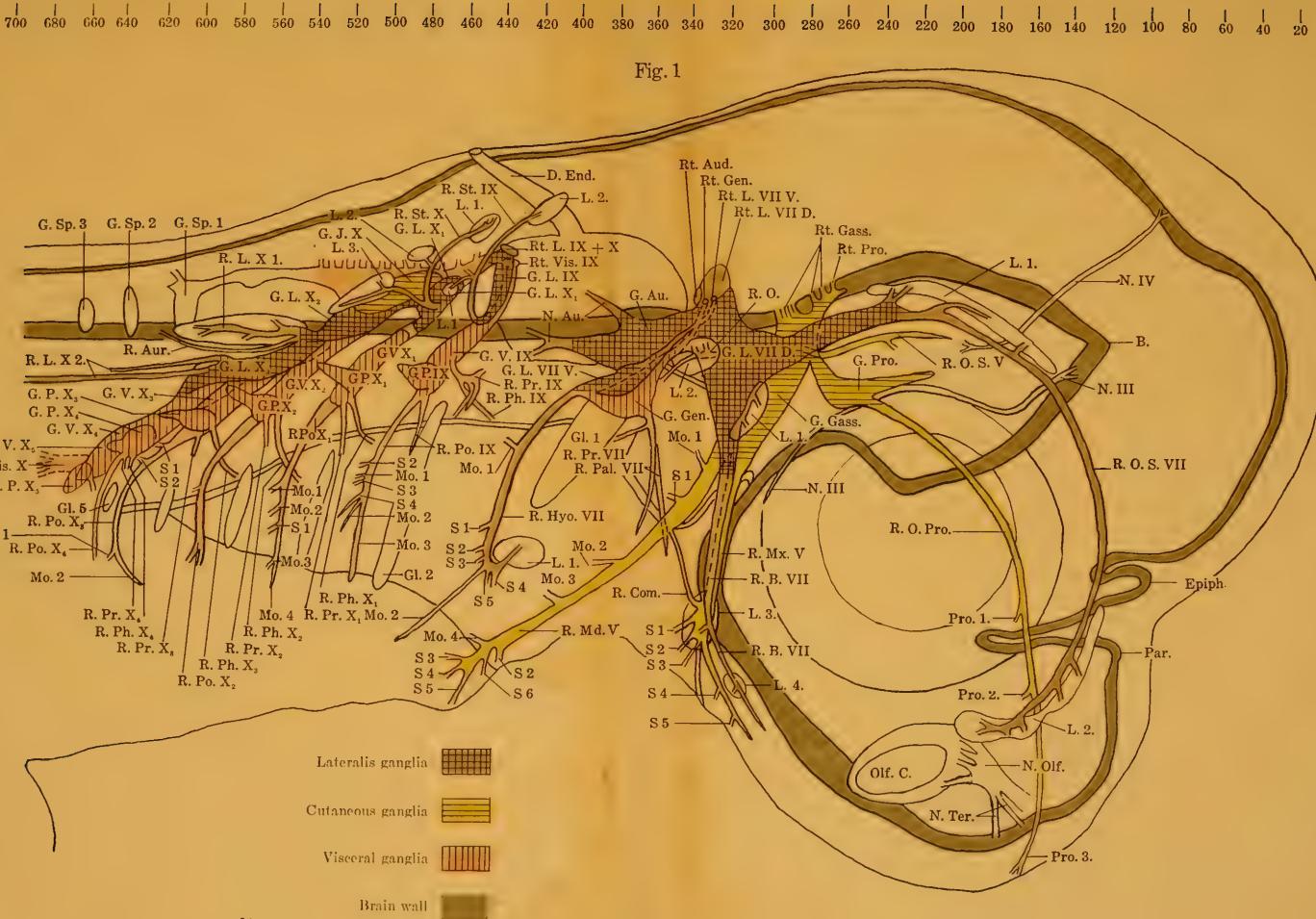
Fig. 1

2 to 13   Camera drawings of transverse sections of the same specimen plotted in figure 1.   The drawings are made at a magnification of 38 and reduced to 25 for publication.   The sections were 10 microns thick for all figures.   The section numbers are to be identified on figure 1.

2   Camera drawing of section 249, which lies just anterior to the root of the profundus ganglion and near the middle of that ganglion.

3   Taken from section 290 at the level of the root of the Gasserian ganglion.

4   Taken from section 321 at the level of the root of the dorsal lateralis ganglion of VII.

5   Taken from section 360 at the level of the origin of r. palatinus VII from the geniculate ganglion.

Fig. 2

R. O. S. VII
R. O. S. V
B. V.
G. Pro.
N. III
H. C.

Fig. 3

R. O. S. VII
No.
G. Gass.
B. V.
M.
N. III
H. C.
B. V.

Fig. 4

G. L. VII D.
No.
B. V
R. Md. V
Hyp.
L.
R. B. VII
R. Mx. V
H. C.
M.
R. Mx. V
R. B. VII

Fig. 5

Au. Ves.
B. V.
G. Au.
G. L. VII V.
No.
G. Gen.
R. Pal. VII
B. V.
M.
R. Md. V
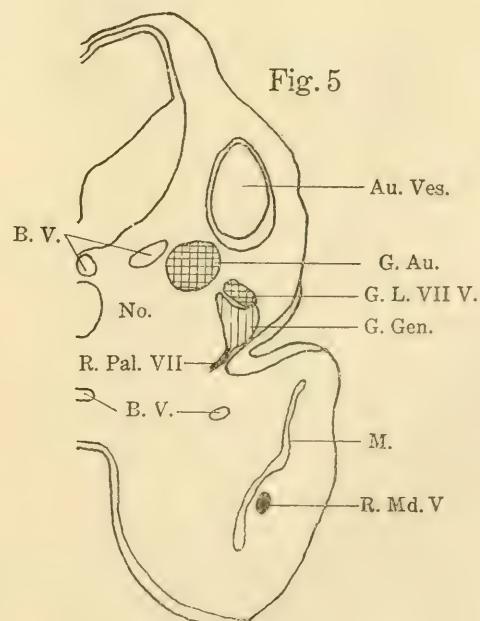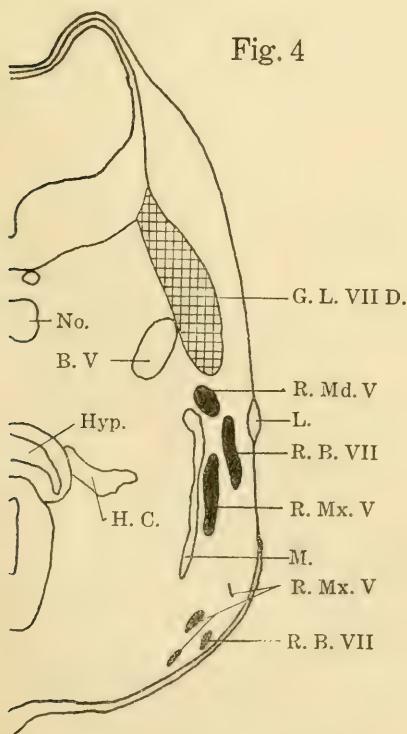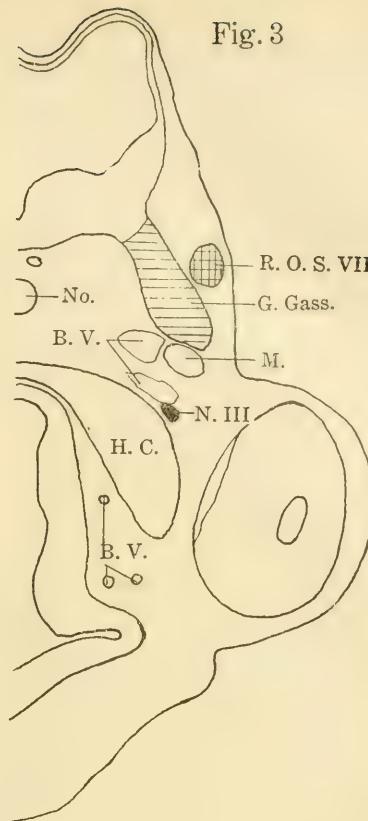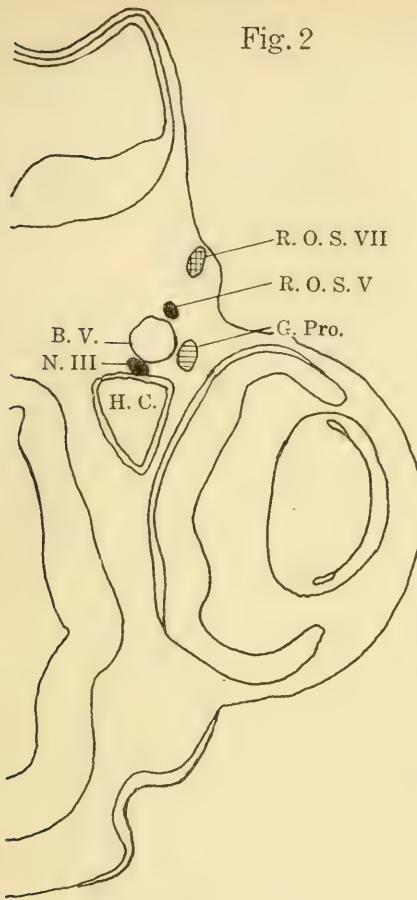
PLATE 3

6 Taken from section 455, is at the level of the origin of the r. supra-temporalis IX from the lateralis IX ganglion. It also passes through the anterior end of the ganglion petrosum, or visceral IX.

7 Taken from section 482, is at the level of the origin of the r. supra-temporalis X from the ganglion laterale X_1 and also passes through the point of origin of the r. posttrematicus IX from the ganglion viscerale IX which is attached to the epibranchial placode at this point.

8 Taken from section 526, is at the level of the origin of the r. pretre-maticus X_1 from the ganglion viscerale X_1 and passes through the placodal attachment of that ganglion.

9 Taken from section 546, lies just posterior to the point of origin of r. lateralis X–1, which arises from the ganglion laterale X_2, and of the nerve identified as r. auricularis X. This section passes through the anterior end of g. laterale X_3.

Fig. 6

Rt. X.
Au. Ves.
R. St. IX
G. L. IX
G. V. IX
R. Pr. IX
R. Ph. IX
No.
B. V.
Ph.
M.
R. Hyo. VII
M.
R. Md. V

Fig. 7

Rt. X.
G. J. X
R. St. X
G. L. X₁
No.
B. V.
G. V. IX + Pl.
R. Po. IX
Ph.
B. V.
M.
R. Md. V

Fig. 8

Rt. X.
L.
Rt. X 2, 3, 4, 5
G. L. X₂
No.
G. V. X₁ + Pl.
R. Pr. X₁
M.
B. V.
Ph.
B. V.

Fig. 9

Rt. X.
L.
R. L. X 1.
R. Aur.
Rt. X 3, 4, 5
G. L. X₃
No.
G. V. X₂
M.
B. V.
R. Po. X₁
Ph.
M.
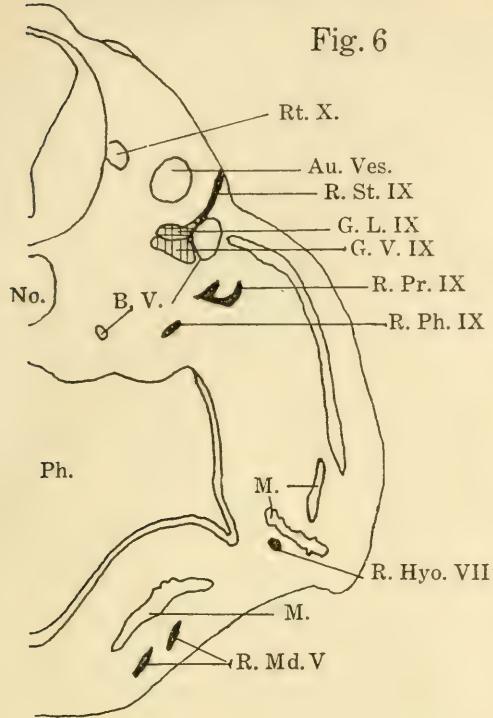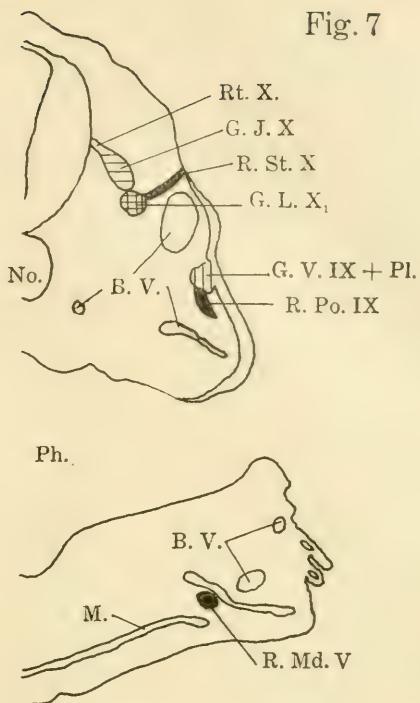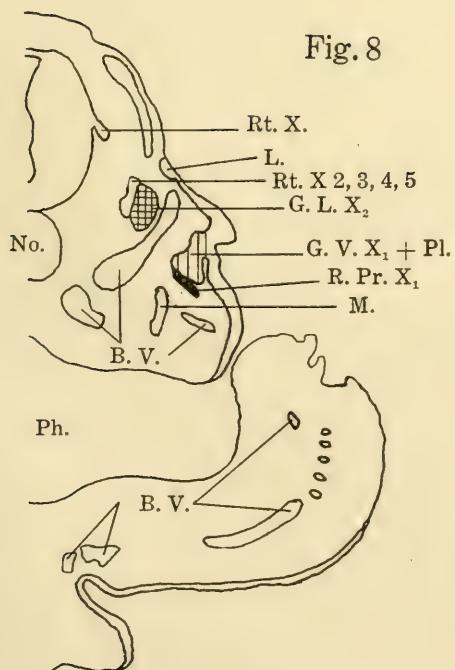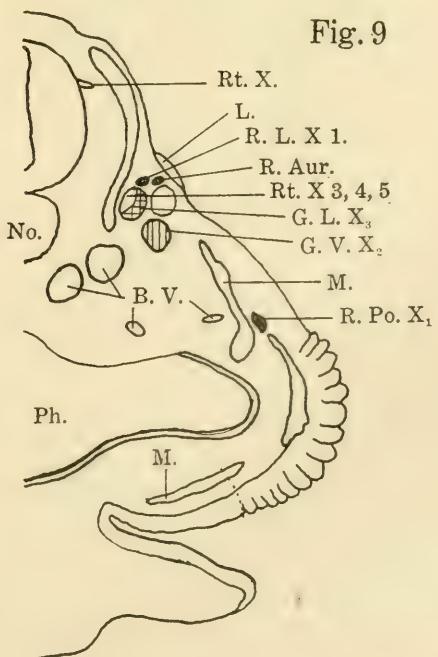
10   Taken from section 569, is at the level of the origin of the r. post-trematicus X_2. It also passes through the middle of the ganglion laterale X_3 and through the extreme anterior end of the ganglion viscerale X_3.

11   Taken from section 596, is at the level of the origin of the r. pretrematicus X_3 from the ganglion viscerale X_3. This is just posterior to the point of fusion of this ganglion with its epibranchial placode. This section passes through the posterior end of the ganglion laterale X_3.

12   Taken from section 628, passes through the fusion of ganglion viscerale X_4 with the ectoderm which is contributing cells to the ganglion at this point.

This section also passes through the anterior end of the ganglion viscerale X_5. The division between ganglia visceralia X_4 and X_5 is shown better in this section than in the reconstruction in figure 1.

13   Taken from section 647, passes through the posterior end of the ganglion viscerale X_5 but anterior to its attachment to its epibranchial placode.

Fig. 10

Rt. X.
M.
R. L. X. 1.
R. Aur.
G. L. X₂
G. V. X₃
No.
G. V. X₂ + Pl.
B. V.
R. Po. X₂
Rt. X 3, 4, 5
B. V
Ph.
B V
B. A.

Fig. 11

Rt. X.
M.
R. L. X. 1.
R. Aur.
No.
G. L, X₃
Rt. X 3, 4, 5
G. V. X₃ + Pl.
R. Pr. X₃
B. V.
M.
R. Po. X₂
Ph.
At. B. V.
B. V.
B. A.
M.

Fig. 12

M.
G. Sp. 2
N. Sp. 1
No.
R. L. X 2.
Rt. X 4, 5
G. V. X₅
G. V. X₄ + Pl.
B. V.
R. Po. X₃
Ph.
M.
At.
B. V.
B. A.
M.

Fig. 13

M.
N. Sp. 3
No.
R. L. X 2.
N. Sp. 2
G. V X₅
B. V.
Ph.
B V
R. Po. X₄
At.
M.
At.
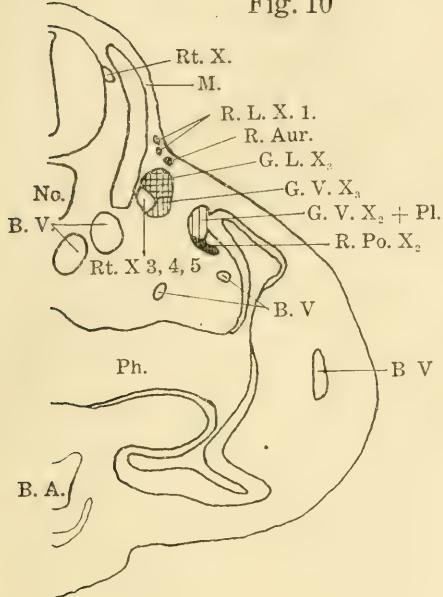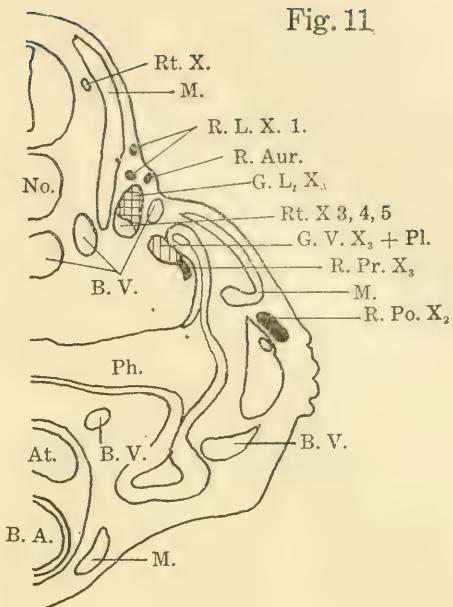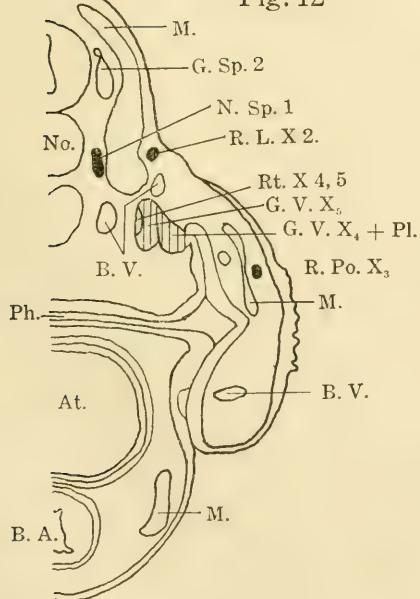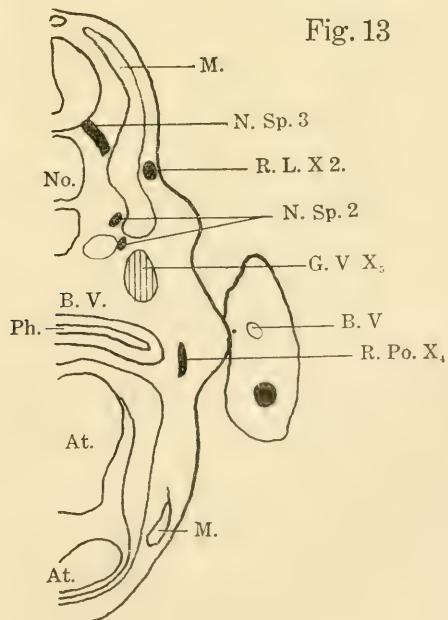
NUCLEAR SIZE IN THE NERVE CELLS OF THE BEE DURING THE LIFE CYCLE

W. M. SMALLWOOD AND RUTH L. PHILLIPS

(*From the Zoological Laboratory of Syracuse University, C. W. Hargitt, Director.*)

ONE FIGURE

The following study of nuclear size in the nerve cells of the antennal lobe of the bee was undertaken for the purpose of learning what are the normal conditions and what, if any, changes they undergo during the life cycle.

Bees afford exceptionally good material for such work because all members of a given swarm are of identical parentage; all spend an inactive larval existence, and the life cycle of individuals varies according to type and season. Drones live through the summer, queens may live for seven years, and the workers, with which we are concerned in this paper, have a life cycle varying from about six weeks in the summer to about six months for the insects hatched from an autumn brood.

Hodge[1] ('92) published his observations on daily fatigue in the bee, the sparrow and the cat. In this work he chose the cells of the antennal lobes because they are easily located. We have limited our study to the cells of this region for the same reason. It is usually considered that excessive stimuli in the form of an immense amount of normal daily work, electrical stimulation, or surgical shock result in a decrease of nuclear size among the nerve cells. That such assumptions are commonly held, the work of Crile[2] and Hodge shows.

Conklin[3] ('12) has shown that there is a normal relation between the size of a cell and its nucleus, and Kocher[4] ('16) has

[1] Journal of Morphology, vol. 7, 1892, p. 153.
[2] Journal of the American Medical Association, vol. 57, no. 23, 1911, p. 1812.
[3] Journal of Experimental Zoölogy, vol. 12, 1912, p. 1.
[4] Journal of Comparative Neurology, vol. 26, no. 3, 1916.

questioned the results obtained by Hodge and Crile. Our work was begun in 1910–11, but the opportunity for completing it did not present itself until this summer. We have re-examined our earlier work and supplemented it with additional material collected and prepared in the same way as that obtained previously.

This material consists of the following stages covering the life cycle of the honey bee.

1. Recently hatched larvae.
2. Half-grown larvae.
3. Fully-grown larvae.
4. Early pupae.
5. Mid-pupae.
6. Late pupae.
7. Newly hatched adults.
8. Young adults taken at 6.30 a.m.
9. Young adults taken at 6.30 p.m.
10. Old adults taken at 6.30 a.m.
11. Old adults taken at 6.30 p.m.
12. Adults taken at close of the winter season.

Several different fixatives were tried, but the only ones found successful were osmic sublimate, 1 per cent osmic acid, 1 per cent glacial acetic, and sublimate to saturation, Carnoy's and Ohlmacher's fluids. Only one individual, that one of stage (8), included in our study was fixed with osmic sublimate.

No attempt was made to dissect out the brains of the larvae, which were embedded entire. The brains of pupae and adults were excised. Sections were cut from four to seven micra thick in paraffin of 54°, and stained in iron haematoxylin with Bordeaux red as a counter stain.

The Zeiss and Leitz eyepiece micrometers were used, readings being computed in micra. We tried to use the planimeter in our work this summer, but found it impracticable in measuring such small nuclei.

There are according to Kenyon,[5] four general regions in the brain of the bee; the dorso-cerebron, the ventro-cerebron, and

[5] Journal of Comparative Neurology, vol. 6, 1896.

the deuto-cerebron or antennal lobes. These latter arise from the ventro-anterior side of the dorso-cerebron by two stalks of fibrillar substance. Each stalk expands into a convoluted spherical mass of fibers from which the nerves of the antennae arise. This fibrillar core is surrounded by nerve cells. In the adult these cells are of three types as far as nuclear size is concerned, which conform to the types described by Kenyon. These are, multipolar giant cells, large and small ganglion cells. In the larva and pupa we find large neuroblasts which give rise to the cells of the last two types by mitosis and finally themselves transform into the giant cells of the adult.

It is manifestly impossible to measure all the nuclei in any ganglion in such a study as this. We must be content to choose and select with as much care as possible, such cells as appear to belong in the same general group and from a study of their measurements attempt to gain some insight into the problems which concern the whole mass of cells. Such cells in each class were chosen as appeared to be fair representatives of the respective groups. It is probable that others in going over the same material would select and measure other cells and so arrive at average measurements somewhat different from those given in our tables. Our experience leads us to believe, however, that the general form of the curves derived from a study of the data would not be materially altered.

Usually we have found no difficulty in making a decision as to the group in which any particular cell belongs. There have been a few instances, however, where the mere matter of size seemed to be insufficient to control the matter of classification. In such cases we have taken into consideration the general appearance of the cells, both as to nucleus and cytoplasm, before placing the cell in one or another group. In the case of the giant cells care was taken to choose those in which the plane of section passed approximately through the center of the nucleus.

Each nucleus was measured in its longest and shortest diameter and the average of these taken as the mean diameter. The results of these measurements are summed up in the following table which gives the average nuclear diameter for the three

Table showing average nuclear diameter, number of cells measured and range of variation for each stage of the life cycle of the honey bee

STAGE[1]		1	2	3	4	5	6	7	8	9	10	11	12
I	Average nuclear diameter	9.05	9.89	9.5	8.91	9.75	9.43	9.76	9.26	9.24	9.76	9.59	9.45
	Number of cells	50	38	36	45	45	38	42	39	41	41	40	40
	Range of variation	7 to 12.9	7 to 12.5	7.45 to 13.3	7.45 to 11.65	7.67 to 13	7.7 to 11.75	7.45 to 12.08	7.5 to 12	7.45 to 11.23	7.9 to 12	8 to 11.05	7.9 to 11.5
II	Average nuclear diameter	5.67	6.52	6.29	7.39	7.06	6.99	6.95	6.85	6.69	7.06	7.04	7.04
	Number of cells	44	45	46	50	53	40	40	39	40	41	46	43
	Range of variation	4.42 to 7.5	5.4 to 8.5	5.4 to 8	6 to 9.25	6 to 8.3	6 to 8.5	6 to 8.25	6 to 8.75	5.4 to 8	6 to 8.25	5.8 to 9.25	5.5 to 8.3
III	Average nuclear diameter	4.44	4.36	4.68	5.23	5.27	5.01	5.06	4.8	4.88	5.29	4.95	4.3
	Number of cells	45	45	46	50	45	50	43	40	42	40	44	41
	Range of variation	3.68 to 5	3.3 to 6	4 to 6	4 to 6.2	3.68 to 6.02	3.4 to 6.2	4 to 6	3.5 to 6.05	4 to 6	4 to 6	4 to 6.05	2.8 to 6

[1] The stages indicated by the Arabic numerals are, (1) Recently hatched larvae; (2) Mid-larvae; (3) Late larvae; (4) Early pupae; (5) Mid-pupae; (6) Late pupae; (7) Newly hatched adults; (8) Young adults taken at 6.30 a.m.; (9) Young adults taken at 6.30 p.m.; (10) Old adults taken at 6.30 a.m.; (11) Old adults taken at 6.30 p.m.; (12) Adults taken in April after wintering. Roman numerals refer to the types of cells measured, I, representing the giant cells; II, the large unipolar cells; and III. the small unipolar cells.

types of cells in each stage, the number of cells measured and the range of variation for each. The results are shown graphically in the curves which follow the table.

A study of these results together with the plotted curves indicates a number of fundamental conditions. In all of the stages in each of the three groups of cells measured, there is a wide range of variation in the size of the nucleus. This cannot be charged to the normal swelling of the nucleus just before mitosis, for the same variation is present in the cells of the bee that had lived through the winter and in the queens studied. That variation is an ever-present condition in all living things is a truism, but when we attempt to indicate which organ or tissue is responsible for the variation most of the observations have been simply a record of the organic fact of variation. This study claims that the cells and their parts such as the nuclei are the variable factors that are responsible for the variation in the tissue or organ. Any explanation of the cause of such variations has to recognize the part played by cells. It seems to the writers that this natural and normal variation plays an important part in explaining such conclusions as Crile comes to in regard to the effects of shock. Before we can accept his conclusions, we must determine what is the normal range of variation for the group of cells that he studied. It would have been a relatively easy problem to indicate a definite tendency beginning with young adults and passing to the winter bee by simply taking some of the large cells in the young adult with nuclear diameter of 12 micra and comparing them with those that measure 7.9 micra. This would give a definite shrinkage with age; but when the average of some forty cells is taken, the total is 9.26 micra for the young adult, and 9.45 for the winter bee. We interpret the difference to be due to the normal variation present in these cells and do not regard the larger average for the winter bee in nerve cells of type I as a measure of the extent of change that as come with fatigue or age.

The second inference to be drawn from these measurements is the independent sequence of growth changes in these three types of nerve cells. There is a more or less rhythmic variation

in the series of measurements made when the plotted curves are
viewed as a whole. Take the large multipolar cells which start
in with an average diameter of 9.05 micra; then increase to
9.89 during the mid-larva period, to be followed by a marked
decline to the mid-pupa period. This is followed by an increase
which is almost the same as the newly hatched adult and the
old adult taken at 6.30 a.m. The average nuclear diameter of
the winter bee is larger than the recently hatched larvae,
late larvae, early pupae and young adults taken early in the
morning. A similar study of the variations in nerve cells of
Type II indicates a different series of growth sequences. Here
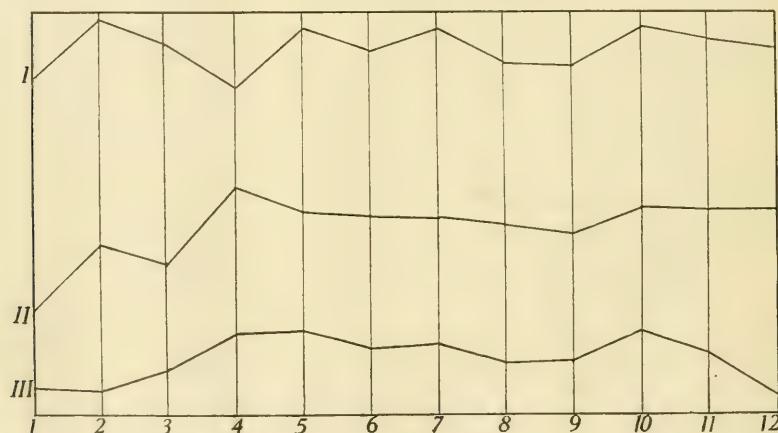
the largest nuclear diameter is during the early pupa stage with
no marked variation in the average until the old adults are
reached. The averages for these three types of cells seems to
us to indicate that there is a definite series of growth sequences
that follow through the life cycle in the worker bee and that
they are not dependent on each other.

Beginning with the old adults taken at 6.30 a.m., there is a
noticeable decrease in nuclear size in cells of Types I and III in
the two following stages studied (11 and 12 of table) that is sim-
ilar to Hodge's results. But there is a more marked decrease
in nuclear size in Type I from mid-larva to early pupa. A simi-
lar change is indicated in the cells of Type II. If the change

in nuclear size in old adults is due to fatigue or old age or both, how are we to explain the larval and pupal change? The larvae of the bee are inactive so that they are more like the pupa than is usual in insects. Metabolism is very active during the larval period but this can hardly result in fatigue to nuclei in the brain. One would expect that the changes that take place during the metamorphosis in the pupa would drain heavily on the energy of the insect, and yet the same rhythmic nuclear changes in size continue through this period. So far as activity is concerned these stages give us the two extremes, the larvae and pupae at rest, the adults of summer extremely busy and the winter workers relatively inactive. If there is a definite nuclear change with work, the stages selected should give us some indication of it. In place of definite nuclear change with age, we find a constant variation which tends to be rhythmic.

CONCLUSIONS

1. In the honey bee worker there is a definite variation in the nuclear size of the nerve cells studied.

2. Changes in nuclear size dependent on the life cycle are unlike in cells of different type.

3. The changes in nuclear size can not be explained as due to the effect of old age or fatigue.

A REVISION OF THE PERCENTAGE OF WATER IN THE BRAIN AND IN THE SPINAL CORD OF THE ALBINO RAT

HENRY H. DONALDSON

From the Wistar Institute of Anatomy and Biology

ONE CHART

In four studies previously published (Donaldson '10, '11, '11 a and '11 b), the percentage of water in the central nervous system of the albino rat has been considered. A revision of this topic was thought necessary, however, for several reasons. In the first place, I wished to record the observed values on which had been based an extensive table giving the percentage of water in the brain and spinal cord according to age, each entry in this table giving also the weight of the brain, or spinal cord to be expected. This table 74, from The Rat (Donaldson '15)—is given in full as an appendix to the present paper. Further, I wished to state the conditions to be observed in the use of this table. In the second place, a statement previously made, required correction:—It has been stated (Donaldson '10) that at a given age the percentage of water in the brain or spinal cord is not significantly modified by the absolute weight of the organ. This conclusion is incorrect, for more careful study shows that weight is a factor in modifying the percentage of water. The evidence for such a conclusion will appear in the course of this paper.

It is the main purpose of the present study, then, to show how the percentages of water, which appear in the appended table 74, have been obtained, and, incidentally, to show how new data must be treated when they are to be corrected for weight. In considering the main points, the brain and the spinal cord will

be treated separately, and for each there will be given in special tables the observed values for the percentage of water, followed by the values corrected for weight, and finally by the formula (or table) values. Using the uncorrected values, the measures of variability in this character will also be given.

THE BRAIN

The data and their treatment

The data used for table 74 (Donaldson '15) have been gradually accumulated during the past eight years, and were collected by random sampling, mainly from the rat colony at The Wistar Institute. The animals used represent, therefore, the general rat population of the colony living, at successive periods, under somewhat varying dietary conditions. In many cases, the rats were small for their age. Although a number of litters are included in the series, the majority of the animals were not closely related to each other.

The records forming this series run from birth to 365 days of age, and may be grouped as follows:

　　　572 male　brains—comprised in 61 age groups
　　　375 female brains—comprised in 61 age groups

About one-half of the records here used were collected for an earlier study (Donaldson '10), and the remainder have been gathered since that time.

The method of removing and preparing the brain and the spinal cord has been described earlier (Donaldson '10). Most of the removals were made by my colleague, Dr. Hatai, and the procedure has been uniform. The drying was done in a large water bath at the temperature of 92°–97°C. We have no reason to think that the observational errors have been important under these conditions.

The accompanying chart 1 shows, for the males only, the course of the percentage of water from birth to 365 days, in both the brain and the spinal cord. The continuous graphs are based on the formulas (pp. 104–105) and the separate entries give the observed values—corrected for weight—for the percentage of
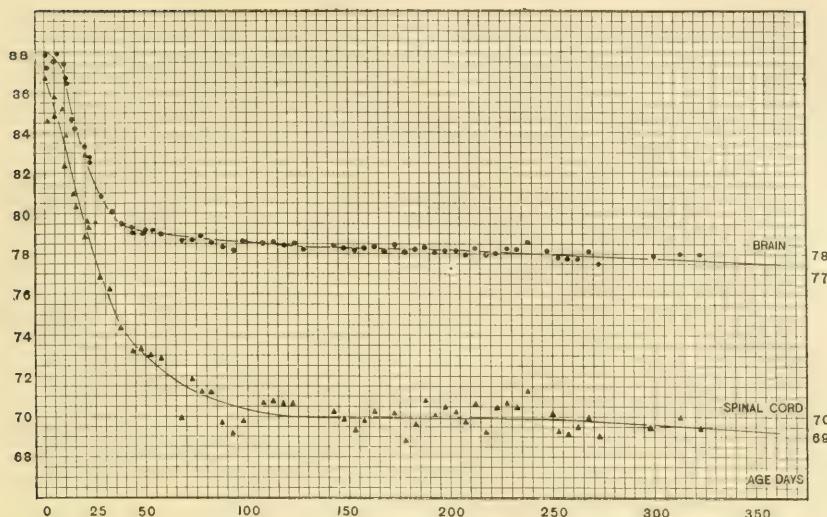
Chart 1 Showing the percentage of water on age in the central nervous system of the albino rat. The upper graph gives the values for the water in the brain as determined by the formulas (Hatai—in 'The Rat,' Donaldson, 1915). The lower graph gives the corresponding values for the spinal cord, determined in the same way. The small black dots indicate for the brain the corrected (observed) values for the several age groups, and these corrected values form the data on which the formulas have been based. The small black triangles have a like significance in relation to the spinal cord.

water in the several age groups, as these appear in table 1, for the brain, and table 2, for the spinal cord.

If we take the mean of the deviations of all of the corrected (observed) values for the several age groups from the corresponding formula values for the percentage of water, as given in table 1, and shown in chart 1 (males only), we obtain the following:

<div style="text-align:center">

Mean of deviations—Males   ±0.19 per cent

Mean of deviations—Females ±0.18 per cent

</div>

Thus it appears that for both sexes the observed values, when corrected for brain weight, differ on the average approximately ±0.2 per cent from the corresponding formula values. Consequently new observations on *random samples*, which fall, after correction, within ±0.2 per cent of the formula values

TABLE 1

*Giving for the percentage of water in the brain the data used for the making of table
74 (Donaldson '15), which is appended to this article. The records are entered
by age groups, male and female records being given separately. For any age
group of either sex the table gives the age to a day—or within a range of five days,
followed by the number of cases—and then by the percentage of water. This datum
appears first, as observed, second, as corrected for the brain weight and third, as
given in table 74, where the values have been computed by formulas (Hatai), these
formulas, in turn, being based on the corrected values as here entered. When the
age is given within a range of five days the interval 5–10 is taken as 8, and 0–5 as
3; i.e., 25—30 = 28 days, 30—35 = 33 days*

AGE IN DAYS	MALES				FEMALES			
	No. of Cases	Percentage of water			No. of Cases	Percentage of water		
		Observed	Corrected	In table		Observed	Corrected	In table
0	20	87.84		88.00	2	89.00		88.00
1	5	87.31		87.95	3	87.62		87.95
5	3	87.43		87.79	4	87.82		87.79
6	3	87.67		87.70	4	87.95		87.70
9	5	87.57		87.05	2	86.70		87.05
10	21	86.79		86.72	2	86.35		86.72
11	5	86.49		86.26				
14	2	84.45		84.97				
15	4	84.23		84.58				
17					2	83.41		83.82
19	5	83.28		83.12				
20	13	82.68	82.91	82.80	3	82.11	82.37	82.82
21	9	82.69	82.87	82.49	3	82.42	82.50	82.51
22	5	82.52	82.67	82.19				
25–30	25	80.58	80.80	80.72	5	80.63	80.60	80.74
30–35	9	79.97	80.10	79.91	3	80.09	80.34	79.94
35–40	27	79.45	79.55	79.46	3	79.72	79.61	79.49
40–45	11	79.23	79.20	79.32	4	79.54	79.37	79.35
45–50	23	79.23	79.17	79.22	20	78.93	78.79	79.25
50–55	27	79.30	79.23	79.14	22	79.34	79.22	79.18
55–60	24	79.05	79.02	79.05	8	79.16	79.13	79.09
60–65					3	79.19	79.04	79.01
65–70	2	78.71	78.59	78.90	4	79.04	78.73	78.94
70–75	9	78.85	78.68	78.84	7	79.06	79.00	78.88
75–80	12	79.11	78.90	78.77	12	78.97	78.71	78.82
80–85	10	78.61	78.52	78.72	9	78.66	78.60	78.77
85–90	8	78.66	78.33	78.67	5	79.08	78.67	78.72
90–95	4	78.44	78.11	78.62	4	78.80	78.70	78.67
95–100	14	78.94	78.63	78.57	17	78.69	78.51	78.62
100–105								
105–110	5	78.44	78.47	78.48	3	78.03	78.03	78.53

TABLE 1—Continued

AGE IN DAYS	MALES				FEMALES			
	No. of Cases	Percentage of water			No. of Cases	Percentage of water		
		Observed	Corrected	In table		Observed	Corrected	In table
110–115	24	78.59	78.53	78.44				
115–120	7	78.53	78.43	78.40	6	78.58	78.37	78.45
120–125	10	78.49	78.53	78.36	3	78.24	78.08	78.42
125–130	10	78.30	78.16	78.33	13	78.39	78.21	78.39
130–135					4	78.28	78.12	78.35
135–140					7	78.68	78.55	78.32
140–145	27	78.48	78.35	78.23	13	78.57	78.39	78.29
145–150	22	78.48	78.24	78.20	10	78.45	78.21	78.26
150–155	8	78.25	78.09	78.18				
155–160	22	78.34	78.23	78.15	14	78.23	78.09	78.21
160–165	11	78.45	78.34	78.13	7	78.43	78.32	78.19
165–170	9	78.22	78.09	78.12	10	78.24	78.15	78.18
170–175	2	78.78	78.44	78.12	4	78.63	78.46	78.18
175–180	5	78.29	78.02	78.11	14	78.28	78.13	78.17
180–185	7	78.29	78.13	78.11				
185–190	4	78.19	78.18	78.11	2	78.58	78.56	78.17
190–195	5	78.14	78.01	78.11				
195–200	9	78.14	78.08	78.10	6	78.14	78.12	78.17
200–205	7	78.24	78.10	78.10	8	78.34	78.16	78.16
205–210	8	78.11	77.97	78.10	4	78.09	78.02	78.16
210–215	8	78.34	78.26	78.09	3	78.40	78.30	78.16
215–220	3	78.06	77.91	78.08				
220–225	2	78.28	78.04	78.07	12	78.23	78.17	78.14
225–230	8	78.31	78.28	78.06	4	78.35	78.16	78.13
230–235	8	78.16	78.20	78.05	2	78.70	78.39	78.12
235–240	4	78.29	78.55	78.04	8	78.26	78.20	78.11
240–245					9	78.36	78.25	78.10
245–250	6	78.29	78.21	78.02	2	78.16	78.09	78.09
250–255	4	78.03	77.85	78.00				
255–260	2	78.08	77.81	77.99	6	78.30	78.25	78.06
260–265	2	78.05	77.75	77.98	2	78.14	77.99	78.05
265–270	2	78.45	78.13	77.96	5	77.86	77.84	78.03
270–275	6	77.85	77.47	77.94				
275–280								
280–285								
285–290								
290–295					3	77.88	78.15	77.95
295–300	4	78.09	77.90	77.85	4	78.31	78.58	77.93
300–305								
305–310					4	77.42	77.54	77.89

TABLE 1—Concluded

AGE IN DAYS	MALES				FEMALES			
	No. of Cases	Percentage of water			No. of Cases	Percentage of water		
		Observed	Corrected	In table		Observed	Corrected	In table
310–315	2	78.04	78.06	77.79				
315–320					4	77.59	77.53	77.84
320–325	4	78.24	77.99	77.74	5	77.92	77.81	77.82
325–330								
330–335					2	77.95	77.90	77.77
335–340					7	78.09	77.89	77.74
340–345	·				3	77.71	77.62	77.71
345–350								
350–355								
355–360					5	77.83	77.62	77.62
360–365					6	77.64	77.49	77.59

may be considered as in agreement. Where one is making comparisons within a litter or within a homogeneous series, less deviation is to be expected and agreement may be limited to values that fall within ±0.1 per cent of the standard which is used. Where data from test animals are contrasted with those from controls of the same litter deviations of 0.05 per cent, if constant or nearly so, may be regarded as significant.

Thus far nothing has been said of the way in which the factors for correction were obtained or how they have been applied. These questions will now be considered.

Sources of variations in the percentage of water

If identical in other respects, two brains of the same age should have the same water content. Two brains are, however, never found to be exactly alike even in the terms of our rather crude measurements, and the differences, which we can at present appreciate, fall into two classes, those which are gross, and those which depend on histological structures.

1. Variations due to gross differences. There are at least two possible causes of variation in the water content dependent on gross characters.

a. The amount of fluid in the ventricles. This is, as a rule, negligible in brains more than 25 days old; but in younger brains and especially during the first 10 days, when the ventricles are relatively large, it may be a modifying factor of importance.

b. Variations in the relative weights of the several parts of the brain. If the brain is divided into the stem, the cerebellum, the cerebral hemispheres, and the olfactory bulbs, it is found that the most variable part of the brain is that formed by the olfactory bulbs. At times these may differ from one another by 50 per cent, in two brains of nearly the same total weight, ranging therefore from 4 per cent to 2 per cent of the weight of the entire brain.

The water content of the mature bulbs is high, 82 per cent. If, as an example, we take the water content of the entire brain as 78 per cent, a reduction of the relative weights of the bulbs from 4 per cent to 2 per cent would cause a loss of 0.1 per cent in the water content of the entire brain, thus reducing it to 77.9 per cent.

Variations in the density of the meninges or in the quantities of blood do not appear to have any significant influence.

2. Variations in the water content of the brain due to histological differences. At the same age large rats have absolutely heavier, and small rats absolutely lighter brains. As there is reason to think that in a given mammalian species, the Norway rat for instance, the number of neurons composing the brain is approximately constant, the difference in the size of the entire brain must therefore mean a difference in the *size* of its constituent neurons and not a change in their number. However, under the usual conditions of growth, shortly after birth myelin begins to appear on the axons. It has been shown that myelin is the constituent mainly responsible for the progressive loss of water from the brain (Donaldson, '16), and although its formation is closely correlated with age, it must be considered probable that slight fluctuations in the relative amount of myelin may occur. These fluctuations would produce in turn small changes in the percentage of water observed.

Inspection of the data at hand suggests that size differences of the brain may depend either on a mere magnification or reduction of the neurons in a strictly proportional manner or on a disproportional growth caused by a relative excess of white substance, in the heavier brain and vice-versa.

As will be pointed out further on, the least variability in the water content of the brain is found *within* the same litter, and it seems probable that here the differences in the brain size which occur are mainly due to a strictly proportional growth of the neurons. On the other hand, it appears that the brain which is large at a given age has commonly anticipated some of the growth changes whch belong to a later period, and this means that the relative abundance of the myelinated axons has been increased—a change necessarily accompanied by a lowering of the water content. This, the more common relation found between two unrelated rat brains of the same sex at like age, where the larger brain usually has the smaller percentage of water, is considered therefore to be due to the relative excess of myelinated fiber substance in the larger brain. As will be seen, this statement constitutes a reversal of the opinion which I formerly held (Donaldson, '10).

If this corrected opinion is accepted, the next step is to determine the factors to be used for reducing any observation on the percentage of water.

On the relation of the percentage of water to the absolute brain weight

According to our hypothesis the relatively small brain is likely to be retarded in development, i.e., to be a trifle behind the stage characteristic for its age and so to have a less proportion of myelin and therefore a higher percentage of water, while on the other hand the relatively large brain is likely to be precocious and to show as a consequence a lower percentage of water.

The test of this assumption was therefore made by averaging for the first and last thirds or halves of each age group, ar-

ranged, according to increasing brain weight, the observed percentages of water. The corresponding weights for the relatively light and for the relatively heavy brains were also averaged. If it turned out that on the average the higher percentage of water went with the light brain lot, and the lower percentage with the heavy brain lot, the determination was considered 'accordant.' The opposite relation was designated as 'reverse.'

As it seems probable that the myelin is the principal cause of the differences for which correction should be made, it is not advisable to introduce corrections for brains from rats less than 20 days of age, since previous to that age myelination is quite incomplete. On looking at table 1, it will be found that beginning with the 20 day records there are observations for the percentage of water on a number of age groups, which comprise five or more individuals. Of such groups we have used 38 from the male, and 32 from the female records. Each of these groups has been treated as indicated in the sample table which follows. The details of this entire operation are here given.

The data for each individual were arranged according to increasing body weight. After the body weight of each rat was entered the observed brain weight, and after this the brain weight to be expected for the *body weight* (not the age) was also entered, using table 68 (Donaldson '15) for the expected brain weight values. If the observed brain weight is found to be greater than that to be expected from the body weight then the brain is large, and vice-versa.

Using the brain weight values taken from table 68 as the standards, the percentage value of each difference was determined, and this was entered opposite the brain weight to which it applied. These percentage differences were next arranged in regular order from the most minus to the most plus, and of the series thus formed either the first third or first half (in this instance the first three) of the cases was used for comparison with the last third or half.

The three minus cases give an average deficiency in brain weight of 6.2 per cent and show 78.57 per cent of water ob-

Sample table, to illustrate the procedure for obtaining a correction factor for the percentage of water as modified by the brain weight. Albino rat. Male. Brain. Age group 210–215 days.

OBSERVED		TABLE 74 VALUES BRAIN WEIGHT	DIFFERENCE, GRAMS		DIFFERENCE IN PER CENT OF TABLE VALUE	
Body Weight	Brain Weight		−	+	−	+
grams	*grams*	*grams*				
160	1.711	1.807	0.096		5.3	
171	1.707	1.824	0.117		6.5	
216	1.982	1.886		0.096		5.0
224	1.765	1.895	0.130		6.9	
251	1.911	.1.925	0.014		0.7	
273	2.197·	1.947		0.250		12.8
276	2.215	1.948		0.267		13.7
		Ave. 1.890				

ARRANGEMENT OF PERCENTAGE DIFFERENCES IN BRAIN WEIGHT FROM MINUS TO PLUS		CORRESPONDING PERCENTAGES OF WATER OBSERVED	
−	+	−	+
First three $\begin{cases} 6.9 \\ 6.5 \\ 5.3 \end{cases}$		77.9 78.7 79.1	
Ave. 6.2		Ave. 78.57	
	Last three $\begin{cases} 5.0 \\ 12.8 \\ 13.7 \end{cases}$		78.1 78.0 78.4
	Ave. 10.5		Ave. 78.17

served. Similarly the three plus cases give an excess of brain weight of 10.5 per cent and show 78.17 per cent of water observed. The relatively heavier brain group has, therefore, the less percentage of water and the relation is 'accordant.'

If we use the mean of the table values for brain weight— 1.890—as the standard and calculate the absolute difference for the brain weights, between the minus group (= −6.2 per cent) and the plus group (= +10.5 per cent) we find this to be 315 mgm. This difference 315 mgm. corresponds to a difference in the percentage of water of (78.57 per cent −78 17) =0.40

per cent so that a difference of 1 mgm. of brain in this group corresponds to a difference of 0.0012 in the percentage of water.

Factors for correcting the percentage of water according to brain weight

The result of treating the data in this manner was to show that of 38 male groups 64 per cent, and of 32 female groups 66 per cent were accordant. In the case of each age group further calculations were made. Taking as the standard the average of all the tabular brain weight values entered, the absolute difference between the average weights of the light and the heavy brains was computed and expressed in milligrams. The difference between the mean percentage of water for the light and for the heavy groups was also found. Then, by dividing this difference in the percentage of water by the difference in weight, expressed in milligrams, the difference for 1 mgm. was found. The number thus found I designate the 'correction factor.' The preceding paragraphs give an example of the foregoing procedure.

Of course, such a factor was obtained for each age group in each sex and was found to be accordant, as stated, in 64 per cent of the male, and 66 per cent female groups, but reverse in the remaining groups. In the case of each sex the sum of the reverse factors was deducted from the sum of the accordant factors and the remainder divided by the number of *accordant* cases. This gave the correction factor for each sex.

The results are as follows:

Correction Factors

Male brain: 0.0013 per cent water for a difference of 0.001 gram
Female brain: 0.0012 per cent water for a difference of 0.001 gram

The correction factor selected for *both* sexes was 0.0013 and this has been used in correcting the observed percentages of water as given in table 1.

The object of this treatment of the crude data was to reduce the deviations in the percentage of water which depended on

differences in the absolute size (weight) of the brain   Therefore by getting the difference in milligrams between the observed brain weight and the brain weight for body weight, as given in table 74, and multiplying this by 0.0013, a correction was obtained which could be applied to the crude values for the percentage of water, which appear in table 1.

From this table there have been omitted, however, both the body weights and the observed brain weights for the several age groups, so that the final results there given cannot be controlled except by reference to the original records which are on file at The Wistar Institute.

Application of the correction factor in the case of new data

To obtain the corrected value for the percentage of water in the brain in the case of a new observation, the necessary data are the body length, the body weight, the observed brain weight, the percentage of water in the brain, and the age and sex of the rat.

It is necessary also to have access to reference tables which give the body weight normal to the body length, and also the brain weight and percentage of water (for that brain weight) normal to the age.

With these data it is possible in a given case first to determine what correction should be made in the observed percentage of water in order to make that value comparable with the percentage of water to be expected when the brain weight was normal to the body length.   This may be illustrated by an example taken from a recent investigation.   The data for the rat selected are as follows:

Body weight, 133.5 grams
Body length, 179 mm.
Age, 173 days—female
Brain weight, 1.581 grams
Percentage of water in brain, 78.61 per cent

If we turn to table 68 in 'The Rat' (Donaldson, '15), it appears that for a female rat 179 mm. long a body weight of 144.4 grams is to be expected.   Therefore this rat is under weight.

Moreover a brain of 1.750 grams should be found with the above body length, but the observed brain weight was only 1.681 grams. It is therefore deficient by 0.069 grams. The observed percentage of water in the brain was 78.61 per cent. The percentage of water to be expected for a female having a body weight of 144.4 grams was 78.62 per cent (table 74, here appended). As the observed brain weight was 69 mgms. too low and the correction factor is 0.0013 per milligram, the total correction amounts to 0.09 which is to be subtracted from the observed value 78.61 per cent, thus giving 78.52 per cent as the corrected value for the percentage of water.

This result may be interpreted as follows: The growth of the brain was retarded in this animal so that although the animal was 173 days old, it had nearly but not quite the water content of a younger rat, the age of which was normal to the body weight.

In view of the fact that we have a series of computed values for the percentage of water found in brains of the *standard size* at various ages, it is possible in this case to determine the probable percentage of water in the brain under examination, if it had reached the size characteristic for its age—173 days.

At this age the brain, according to table 74, should weigh 1.835 grams and have 78.18 per cent of water. This tabular brain weight is 154 mgm. above the observed weight 1.681 grams, with a water content of 78.61 per cent. The difference 154 mgm. multiplied by the correction factor 0.0013 gives a correction of 0.2 which is to be subtracted from the water content observed, 78.61 per cent, giving as the corrected value 78.41 per cent against the tabular value of 78.18 per cent. This shows a deviation from the tabular value of about 0.23 per cent or an amount just outside the range of ±0.2 for random sampling. This somewhat elaborate process seems necessary to reduce the crude data to a form in which they may be compared with each other.

Percentage of water according to sex

When the values given in table 74—here appended—are examined, we note that from the age of 60 days on the body

weights and brain weights of the female are regularly less than
those for the male. If we determine for a series of cases the
difference in the percentage of water between the male and
female brains of like age, we find that a difference of 1 mgm. in
brain weight corresponds to a water difference of about 0.001
per cent, thus giving an amount which is a trifle below the cor-
rection factor for the brain within each sex. It seems prob-
able that the perikarya of the neurons in the male are relatively
somewhat larger than in the female, and this would account
for the slightly lower value found by this method of comparison.

Measures of variability in the percentage of water

The material represented by the data in table 1 makes it
possible for the first time to determine the variability of the
percentage of water in brains of like age, not only when the
brains are taken by random sampling, but also when they be-
long to a single litter. The measures of variability determined
for each sex were the standard deviation (σ) and the coefficient
of variability (C).

For the determination of the standard deviation σ we used
the formula

$$\sigma = \frac{\sqrt{\Sigma (x^2 . f)}}{n}$$

and for the probable error of the standard deviation

$$E_\sigma = \pm 0.6745 \frac{\sigma}{\sqrt{2n}}$$

For the coefficients of variability, C, the formula

$$C = \frac{\sigma}{A} \times 100$$

and for the probable error of the coefficient of variability

$$E_c = \pm 0.6745 \frac{C}{\sqrt{2n}}$$

(Davenport '04).

The uncorrected values for the percentage of water were used in this series, as well as in all of the other series examined. The differences between the results based on the corrected, and those based on the uncorrected values are however negligible.

In the case of the males, as will be seen from table 1, we have from birth to 163 days of age, 19 groups containing 10 or more entries and averaging 18 observations in a group.

From the treatment of this material we obtained the following:

Standard Deviation—Males

Range in 19 groups   0.21±0.028 to 0.50±0.080.
Average of 19 groups   0.32±0.035

Coefficient of variability—Males

Range in 19 groups   0.26±0.034 to 0.64±0.090
Average of 19 groups   0.40±0.047

In the case of the females we have 11 groups averaging 14 individuals in a group and ranging from 48 to 223 days of age. From the treatment of this material we obtained the following:

Standard deviation—Females

Range in 11 groups   0.22±0.030 to 0.45±0.016
Average of 11 groups   0.31±0.040

Coefficient of variability—Females

Range in 11 groups   0.28±0.038 to 0.57±0.077
Average of 11 groups   0.40±0.050

It appears from the foregoing that the variability in the percentage of water is nearly alike in the two sexes, and that it is remarkable small ($\sigma = 0.31$ and $C = 0.40$ per cent), thus supporting the conclusion that normally the water content in the brain is highly constant when taken in relation to age. A number of age groups, used for the preceding determinations, contain records that belong to one or to several litters. It seemed probable that the variability would be less within a given litter than in the mixed population, or in a group composed of all the members of the several litters. Among the 19 male groups, just examined, 9 contained from one to four litters each composed of three or more individuals. In all there were 21 litters

available for examination. The average variability in the 9 groups from which the 21 litters are taken was

$$\sigma = 0.26 \pm 0.029 \text{ and } C = 0.32 \pm 0.035,$$

while the average of the variabilities of the 21 litters, within these 9 groups, was

$$\sigma = 0.14 \pm 0.033 \text{ and } C = 0.17 \pm 0.041.$$

Thus the variability of the male litters is only about one-half that of the age groups in which they are found.

Among the 11 female age groups, there were 7 which contained 11 litters of sufficient size for study. Here we find much the same relations as appeared among the males. The average variability of the 7 female groups from which the 11 litters were taken was as follows:

$$\sigma = 0.25 \pm 0.032 \text{ and } C = 0.32 \pm 0.041$$

while the average of the variabilities of the 11 female litters was:

$$\sigma = 0.13 \pm 0.30 \text{ and } C = 0.17 \pm 0.043$$

Again the litter variability is about half that of the groups from which the litters were taken.

It appears from the foregoing that the variability of the percentage of water in brains belonging to the same age group is small—and that it is about the same for both sexes—but that within a given litter it tends to be much less than in the age group formed by a combination of the litters

THE SPINAL CORD

Although the number of records for the spinal cord is a trifle less than the number for the brain, yet all the spinal cords which were used are from rats that also furnished brains for the brain series. What has been said already (p. 78) in connection with the brain, concerning the material and the general character of the data, applies therefore to the spinal cord series also. In

discussing the data we shall follow the same order of presentation as was followed for the brain. The records for the spinal cord run from birth to 365 days of age and may be grouped as follows:

569 male   spinal cords comprised in 61 age groups
363 female spinal cords comprised in 56 age groups

Thus in the case of the females there are five age groups less for the spinal cord than for the brain.

For the graph representing the course of the loss of water in the cord and the relation of the corrected (observed) male values to those computed, the reader is referred to chart 1, p. 79. If we take the mean of the deviations of all of the corrected values for the percentage of water from the corresponding formula values for the several age groups as given in table 2, and shown in chart 1 (males only), we obtain the following:

Mean of deviations — males $\pm$0.61 per cent
Mean of deviations — females $\pm$0.55 per cent

Thus it appears that the corrected observed values for the water in the spinal cord deviate on the average approximately $\pm$ 0.6 per cent from the corresponding formula values. This deviation is about three times that found for the brain. As a consequence new observations on *random samples* which after correction fall within $\pm$ 0.6 per cent of the formula values may be considered as in agreement. Where one is dealing with very uniform material less deviation is to be expected and agreement may be limited to values that fall within $\pm$ 0.3 per cent of the standard which is used. Where data from test animals are contrasted with those from controls of the same litter deviations of 0.1 of a per cent, if constant or nearly so, may be regarded as significant.

Sources of variation in the percentage of water—spinal cord

The gross differences already noted as modifying the percentage of water in the brain do not apply to the cord, because of the dissimilarity in its architecture; but so far as the differ-

TABLE 2

Giving data for the percentage of water in the spinal cord used for the making of table 74 (Donaldson '15), which is appended to this article. The records are entered by age groups, male and female records being given separately. For any age group of either sex the table gives the age to a day, or within a range of five days, followed by the number of cases—and then by the percentage of water. This datum appears first, as observed, second, as corrected for the spinal cord weight, and third, as given in table 74, where the values have been computed by formulas (Hatai), these formulas, in turn, being based on the corrected values as here entered. When the age is given within a range of five days the interval 5–10 is taken as 8, and 0–5 as 3, i.e., 25 − 30 = 28 days, 30 − 35 − 33 days.

AGE IN DAYS	MALES				FEMALES			
	No. of Cases	Percentage of water			No. of Cases	Percentage of water		
		Observed	Corrected	In table		Observed	Corrected	In table
0	20	86.85		86.75	2	84.80		86.75
1	5	84.71		86.42	3	84.83		86.42
5	3	84.98		85.07	4	85.40		85.07
6	3	85.51		84.73	4	86.31		84.73
9	5	85.46		83.73	2	83.50		83.73
10	21	82.95	82.33	83.40				
11	5	84.14	83.97	82.98				
14	2	81.15	80.78	81.77				
15	4	80.61	80.26	81.39				
17					2	80.56	79.94	80.49
19	5	79.70	79.57	79.90				
20	13	79.39	78.81	79.55	3	78.59	78.24	79.47
21	9	79.84	79.51	79.21	3	78.74	78.39	79.02
22	5	79.58	79.43	78.87				
25–30	25	75.96	76.88	77.00	5	76.02	75.78	76.76
30–35	9	75.37	76.21	75.64	3	74.00	74.04	75.40
35–40	27	73.98	74.36	74.46	3	74.93	74.43	74.26
40–45	11	73.83	73.13	73.74	4	74.08	73.44	73.60
45–50	23	73.95	73.33	73.17	20	73.60	72.72	73.12
50–55	27	73.21	73.03	72.69	22	73.96	73.08	72.69
55–60	24	73.02	72.86	72.27	8	73.49	73.21	72.27
60–65					2	72.69	71.92	71.91
65–70	2	72.35	69.90	71.60	4	73.50	71.73	71.61
70–75	9	72.61	71.71	71.32	7	72.21	71.65	71.36
75–80	12	73.17	71.27	71.09	12	73.20	71.31	71.15
80–85	10	72.36	71.30	70.89	9	72.43	71.56	70.96
85–90	8	72.63	69.63	70.71	5	73.52	71.05	70.80
90–95	4	72.46	69.06	70.56	4	72.80	71.16	70.67
95–100	14	73.00	69.70	70.43	14	73.02	71.66	70.55
100–105								
105–110	5	70.72	70.56	70.23	3	70.16	69.04	70.38

TABLE 2—Continued

AGE IN DAYS	MALES				FEMALES			
	No. of Cases	Percentage of water			No. of Cases	Percentage of water		
		Observed	Corrected	In table		Observed	Corrected	In table
110–115	24	71.68	70.71	70.15				
115–120	7	70.88	70.59	70.09	6	72.31	70.50	70.27
120–125	10	71.71	70.68	70.05	2	71.47	70.61	70.24
125–130	11	71.36	69.40	70.02	13	71.68	70.15	70.22
130–135					4	71.12	68.96	70.22
135–140								
140–145	27	71.62	70.30	70.00	20	72.27	70.64	70.22
145–150	22	72.20	69.95	70.00	10	72.26	70.39	70.22
150–155	8	70.99	69.35	70.00				
155–160	22	71.32	69.95	70.00	14	71.28	69.62	70.22
160–165	10	71.31	70.27	70.00	7	71.65	70.73	70.22
165–170	9	71.16	70.12	70.00	10	71.24	70.10	70.22
170–175	2	72.32	70.70	70.00	4	72.01	70.70	70.22
175–180	5	71.19	68.63	69.99	14	71.35	70.26	70.22
180–185	7	71.14	69.65	69.99				
185–190	4	71.17	70.77	69.99	2	71.51	70.26	70.22
190–195	5	70.96	70.01	69.98				
195–200	8	71.25	70.49	69.97	6	71.25	70.39	70.21
200–205	7	71.07	70.30	69.96	8	71.39	70.31	70.20
205–210	8	70.48	69.72	69.95	4	70.59	69.56	70.19
210–215	8	71.33	70.63	69.93	3	70.76	69.76	70.18
215–220	3	70.34	69.12	69.92				
220–225	2	71.35	70.45	69.90	12	71.33	70.21	70.15
225–230	8	71.45	70.60	69.88	4	72.20	70.70	70.14
230–235	8	70.06	70.42	69.87	2	71.09	69.54	70.12
235–240	2	70.67	71.30	69.85	8	71.13	70.04	70.11
240–245					9	71.49	70.36	70.09
245–250	6	71.31	70.10	69.80	2	70.63	69.81	70.06
250–255	4	71.46	69.26	69.75				
255–260	2	70.92	69.08	69.75	6	71.68	71.07	70.01
260–265	2	70.07	69.45	69.73	2	71.12	69.47	69.99
265–270	2	71.50	69.99	69.71	5	70.89	69.67	69.97
270–275	6	71.23	68.93	69.68				
275–280								
280–285								
285–290								
290–295					3	70.56	70.72	69.85
295–300	4	71.23	69.49	69.55	4	71.50	69.89	69.83
300–305								
305–310								
310–315	2	70.04	70.00	69.46				

TABLE 2—Concluded

AGE IN DAYS	MALES				FEMALES			
	No. of Cases	Percentage of water			No. of Cases	Percentage of water		
		Observed	Corrected	In table		Observed	Corrected	In table
320–325	4	71.02	69.36	69.40	5	70.83	70.14	69.70
325–330								
330–355					2	71.18	71.05	69.64
335–340					7	70.67	69.68	69.61
340–345								
345–350								
350–355								
355–360					5	70.12	68.96	69.48
360–365					3	71.01	69.93	69.45

ences depend on histological composition, the sources of variation for the spinal cord are similar to those for the brain (p. 83).

On the other hand, we have the condition of *adaptation by enlargement* emphasized in the cord, and represented there particularly by the passive lengthening, whereby the cord adapts itself to the varying lengths of the vertebral canal: an adaptation which seems to be accomplished mainly by changes in the quantity of the white substance.

Factors for the correction of the percentage of water according to the spinal cord weight

Theoretically there can be little question that the conditions represented by the *relative weight* (i.e., relative to the body weight) act as in the case of the brain to produce a high percentage of water in the cord which is relatively small, and vice versa. But the cord data cannot be used in the same way as we used the data for the brain, because the absolute weight of the cord is the dominating factor, owing to the fact that the increase in the weight of the spinal cord is so largely due to the addition of myelinated fibers. To obtain correction factors for the cord it has been necessary therefore, to deal with the data from the standpoint of absolute weight. Our assumption is that at the same age the absolutely heavier spinal cord will

have the smaller percentage of water, and vice versa. There were tested 50 male and 37 female age groups. Each of these groups has been treated as follows: The data were arranged according to the increasing spinal cord weights and after each cord weight the percentage of water found in it was set down. Then the averages of the spinal cord weights and of the corresponding percentages of water for the first third or half of the groups were compared with respective averages for the last third or half.

Where the lighter cord was associated with the greater percentage of water, the data were considered as 'accordant,' but where the opposite relation was obtained as 'reverse.' When treated in this way it was found that of 50 male age groups, 84 per cent, and of 37 female age groups, 70 per cent, were accordant. Thus in the cord a heavier weight was associated with a less percentage of water somewhat more frequently than in the case of the brain (p. 87). To obtain the correction factors the difference between the averages for the percentage of water was divided by the number of milligrams by which the corresponding average cord weights differed, and the value for one milligram of cord weight was thus obtained. This gave the correction factor for a single age group. The correction factors to be applied at the different phases of growth were determined arbitrarily by taking the average of the accordant correction factor values in the several age groups within each phase. Two such phases were recognized, as given in table 3.

By the use of the factors thus obtained the corrected percentages of water in table 2 were determined. To obtain these the observed weight of the cord in each age group was subtracted from the cord weights characteristic for that age and this difference in milligrams multiplied by the appropriate correction factor. The observed percentage of water was then corrected by the amount of this product.

It is to be noted that in the case of the cord, in which the myelination process begins during the first or second day after birth, corrections can be applied as early as the tenth day of life.

TABLE 3

Albino rat. Change in the percentage of water for each 0.001 gram of spinal cord weight as obtained from the comparison of the light and heavy cords in the same age group

PHASE	AGE IN DAYS	CORRECTION FACTORS	
		Males	Females
1	10–58	0.010	0.008
2	58–365	0.009	0.007

As noted in the case of the brain, neither the body weight nor the observed cord weight for the several age groups are given. These data, however, have been placed on file at The Wistar Institute.

Application of the correction factors in the case of new data

To obtain the corrected value for the percentage of water in the case of a new observation on the spinal cord, the same data are required as in the case of the brain (p. 88). The details are presented in following paragraphs:

Body length is a more reliable guide than body weight. If we continue the illustration of procedure with the same case as that which was used for the brain (see p. 88) we have as data: body length 179 mm., age 173 days, female, cord weight 0.458 gram, percentage of water 72.00 per cent.

If we compare the observed value with that in table 74 for this age—173 days—it appears that the cord weight expected was 0.580 gram, or 0.122 gram in excess of the observed weight. Using 0.007 as the correction factor for 1 mgm., the total correction amounts to 0.854 to be subtracted from 72 per cent, the observed water content, thus giving the final percentage as 71.15 per cent. Table 74 gives 70.22 per cent for this age, so that when tested in this manner the corrected value is about 1 per cent too high. We conclude in this instance, as we did previously, in the case of the brain, that the growth changes in the spinal cord of this rat had been somewhat retarded.

When the factors for correction in the case of the spinal cord are compared with the single factor for the brain, it is at once evident that those for the cord are much larger.

Although it is not possible to explain this difference in detail or with precision, nevertheless the fact that there should be a difference and one of about the amount found, can be shown readily. In the first place it must be remembered that the correction factors, both for the cord and the brain, have been computed for an absolute weight—0.001 gram. The cord, however, weighs on an average only one-fourth as much as the brain. The relative value of 0.001 gram in the case of the cord is therefore four times that for the brain, and consequently the equivalent factor for correction would be some four times as large as that for the brain.

Further, a study of the formation of the lipoids (Koch, W. and Koch, M. L. '13) shows that the myelination process in the cord is accompanied by the formation of about twice as much lipoid substance as in the brain, and because the lipoid formation is a rough indicator of the formation of myelin sheaths—and these in turn mean a less percentage of water— it follows that the change of 0.001 gram of absolute weight in the cord involving as it does a larger change in the lipoids will for this reason have a greater effect in terms of the percentage of water than does the corresponding change in the case of the brain. This would again increase the correction factor for the spinal cord. Thus, although we cannot justify the factors for the cord in detail, the foregoing considerations indicate that values are to be expected similar to those which have been accepted and used. Further, since the weight of the cord is so largely a matter of white substance the fact that for the female cord—which is typically lighter than that of the male at the like age—the correction factor is smaller, is in accord with the general relation of the white substance as here described.

Percentage of water according to sex

After 60 days of age, a comparison can be made, by the use of the data in table 74, of the weights and water content of the spinal cords in the males and females at like ages. Such a comparison shows the correction factor between the sexes to be about 0.008 per cent of water per milligram. This lies roughly between the correction factor values for the two sexes, as previously determined.

Measures of variability in the percentage of water—spinal cord

As in the case of the brain, it has been possible to obtain for the spinal cord, in a number of age groups of both sexes, the measures of variability as represented by the standard deviation and the coefficient of variability. The formulas used have been given on p. 90. In the case of the males, as will be seen from table 2, we have from birth to 163 days of age 19 groups containing 10 or more entries, and averaging 18 observations per group.

Using the uncorrected values we obtained the following:

Standard deviation

Range in 19 groups 0.74 ± 0.07 to 1.86 ± 0.26
Average of 19 groups 1.06 ± 0.12

Coefficient of variability

Range in 19 groups 0.96 ± 0.09 to 2.64 ± 0.37
Average of 19 groups 1.46 ± 0.17

In the case of the females we have 11 groups, averaging 14 individuals in a group, and ranging from 48–223 days of age. We obtained the following:

Standard deviation

Range in 11 groups 0.60 ± 0.08 to 1.13 ± 0.12
Average of 11 groups 0.81 ± 0.10

Coefficient of variability

Range in 11 groups 0.85 ± 0.11 to 1.56 ± 0.17
Average of 11 groups 1.13 ± 0.14

It is evident from the foregoing that the variability in the percentage of water is somewhat greater in the case of the males than in the case of the females.   When the corresponding mean values for the variability of the brain are compared with those for that of the cord we find that the values for the male cord are about 3.4 times that for the brain and the values for the female cord about 2.6 times.

Among the 19 male groups examined there were 9 which contained from 1 to 4 litters, composed of three or more individuals.   In all there were 21 litters.

The average variability in the 9 age groups in which the 21 litters occurred was

$$\sigma = 0.80 \pm 0.091 \qquad C = 1.05 \pm 0.120$$

while the average of the variabilities of the 21 litters taken from these 9 age groups was

$$\sigma = 0.36 \pm 0.07 \qquad C = 0.46 \pm 0.11$$

Thus among the males the variability within the litters was only about one half of that found for the age groups.   It seems not improbable that myelin formation, which is so much more active in the cord than in the brain, may also be relatively more variable in the cord and thus contribute to the higher variability of this organ in general, and of the cord of the male, in particular.

Among the females there were 7 age groups which contained from 1 to 3 litters composed of three or more individuals.   In all there were 12 litters.   The average variability of the 7 age groups in which the 12 litters occurred was

$$\sigma = 0.72 \pm 0.094 \qquad C = 1.01 \pm 0.13$$

While the average of the variabilities of the 12 litters was

$$\sigma = 0.31 \pm 0.08 \qquad C = 0.42 \pm 0.11$$

Again the litter variability is less than one half of that for the groups from which the litters were taken.

SUMMARY

Evidence has been adduced for the view that both the relative and the absolute weight of the brain and the absolute weight of the spinal cord, at a given age, are factors tending to modify the percentage of water present, in the sense that the heavier brain or cord usually shows the smaller percentage of water.

A presentation has been made also of the data which were used as a basis for the formulas by which the percentages of water in table 74—here appended—have been determined, and of the manner in which the observed values for the percentages of water in the brain and in the spinal cord have been corrected for the weights of the respective organs. Factors for correction have also been given for reducing the observed values for the percentage of water in the brain or cord to a form in which they may be fairly compared with one another or with the values in table 74, when it is desired to use such a table for reference. The factors for correction are given on p. 87 for the brain, and in table 3 on p. 98 for the spinal cord.

It is understood, of course, that when an investigator has homogeneous data, a comparison of these data with one another can be perfectly well made without cross reference to a table such as that here given. On the other hand, where the series of data are from different researches or from different strains of rats they should be referred to such a table before they are compared with each other. The measures of variability have also been found for the percentage of water both in the brain and in the spinal cord, and it has been pointed out that in both organs the variability is small, but that the variability for the cord is about three times that for the brain. Further, it appears that the variability within litters is only about half that found in the age groups to which these litters belong, a relation similar to that already found for the body weight by Jackson ('13) and by King ('15). The measures of variability are given on pages 91–92 for the brain and on pages 100–101 for the spinal cord.

In the appendix are reprinted the formulas for the determination of the percentage of water in the brain and in the spinal

cord, as well as table 74 (Donaldson '15) giving the percentage of water in the brain and spinal cord for the first 365 days of life.

LITERATURE CITED

DAVENPORT, C. B. 1904 Statistical methods with special reference to biological variation. 2d ed., 1904, John Wiley and Sons, New York.

DONALDSON, H. H. 1910 On the percentage of water in the brain and in the spinal cord of the albino rat. Jour. Comp. Neur., vol. 20, no. 2, pp. 119–144.

1911 On the influence of exercise on the weight of the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, no. 2, pp. 129–137.

1911 a The effect of underfeeding on the percentage of water, on the ether-alcohol extract, and on medullation in the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, no. 2, pp. 139–145.

1911 b An interpretation of some differences in the percentage of water found in the central nervous system of the albino rat and due to conditions other than age. Jour. Comp. Neur., vol. 21, no. 2, pp. 161–176.

1915 The Rat. Reference tables and data for the albino rat (Mus norvegicus albinus) and the Norway rat (Mus norvegicus). Memoirs of The Wistar Institute of Anatomy and Biology, no. 6, pp. 1–278.

1916 A preliminary determination of the part played by myelin in reducing the water content of the mammalian nervous system (albino rat). Jour. Comp. Neur., vol. 26, no. 4, pp. 443–451.

HATAI, S. 1915 In 'The Rat.' (Ed. by Donaldson.) Memoirs of The Wistar Institute of Anatomy and Biology, no. 6, pp. 1–278.

JACKSON, C. M. 1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. Jour. Anat., vol. 15, pp. 1–68.

KING, HELEN D. 1915 On the weight of the albino rat at birth and the factors that influence it. Anat. Rec., vol. 9, pp. 213–231.

KOCH, W. AND KOCH, M. L. 1913 Contributions to the chemical differentiation of the central nervous system. III. The chemical differentiation of the brain of the albino rat during growth. J. Biol. Chemistry, vol. 15, pp. 423–448.

APPENDIX

Formulas for the percentage of water in the central nervous system (Hatai, in The Rat, Donaldson '15, p. 170–172).

PERCENTAGE OF WATER IN BRAIN

The formulas do not apply to rats under 10 days of age.

The data for the first 10 days are from direct observations.

Percentage of water in brain—(male) =

$$92.122 - 0.614\ Age + 0.00739\ Age^2\ (Phase\ 1) \qquad (40)$$
$$[10 < Age < 40]$$
$$= 82.756 - 2.103\ log\ Age\ (Phase\ 2) \qquad (41)$$
$$[40 < Age < 160]$$
$$= 77.671 + 0.00537\ Age - 0.000016\ Age^2\ (Phase\ 3) \qquad (42)$$
$$[160 < Age < 365]$$

To transform any determination for the male into that for the female, the value for the male at a given age (see formulas (40) (41) (42)) is modified by a *plus* correction (Hatai).

$$Correction\ (plus) = 0.0555\ log\ (age + 3) - 0.0606 \qquad (42a)$$
$$[10 < Age < 365]$$

The foregoing (40)–(42a) replace the formulas given in the paper by Donaldson ('10).

Formulas (40) (41) (42) (42a) were used for table 74.

PERCENTAGE OF WATER IN SPINAL CORD

The formulas do not apply under 10 days of age. The data for the first 10 days are from direct observations.

Percentage of water in spinal cord—male =

$$87.976 - 0.494\ Age + 0.00364\ Age^2\ (Phase\ 1) \qquad (43)$$
$$[10 < Age < 40]$$
$$= 100.3 + 0.0548\ Age - 17.7\ log\ Age\ (Phase\ 2) \qquad (44)$$
$$[40 < Age < 150]$$
$$= 62.186 - 0.0121\ Age + 4.434\ log\ Age\ (Phase\ 3) \qquad (45)$$
$$[150 < Age < 365]$$

To obtain from the values for the male at different ages the corresponding value for the female, several corrections are required and these differ according to age.

From 10 to 50 days the following correction formula (45a) is used:

$$Correction\ (minus) = 0.0006\ Age^2 - 0.036\ Age + 0.3 \qquad (45a)$$

The values thus obtained are subtracted from the computed values for the males at the corresponding ages.

From 50 to 65 days no correction is made.

From 65 days to 135 days, correction is made according to the formula (45b)

$$Correction\ (plus) = 0.823\ log\ (Age+1) - 0.000542\ (Age+1) - 1.4616$$
$$(45b)$$

From 135 and 165 days the correction is uniform thus:

$$Correction\ (plus) = 0.22 \qquad (45c)$$

From 165 to 365 days correction is made by the following formula:

$$Correction\ (plus) = 0.22 + 0.0005\ (Age - 165) \qquad (45d)$$

The foregoing (43)–(45d) replace the formulas given in the paper by Donaldson, '10.

Formulas (43)–(45d) were used for table 74.

Table 74, which follows, is reprinted from The Rat (Donaldson '15). It gives, for the albino rat, the brain weight and the percentage of water in the brain and in the spinal cord for each sex, on age.

HENRY H. DONALDSON

TABLE 74

Giving the percentage of water in the brain and in the spinal cord for each sex, on age

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
B	4.7	0.217	88.00	0.033	86.75	4.6	0.213	88.00	0.033	86.75
1	5.5	0.290	87.95	0.038	86.42	5.4	0.269	87.95	0.037	86.42
2	5.9	0.333	87.90	0.041	86.08	5.8	0.323	87.90	0.041	86.08
3	6.4	0.395	87.85	0.046	85.74	6.3	0.373	87.85	0.045	85.74
4	6.9	0.442	87.83	0.050	85.41	6.8	0.421	87.83	0.050	85.41
5	7.6	0.509	87.79	0.056	85.07	7.5	0.492	87.79	0.056	85.07
6	8.5	0.581	87.70	0.064	84.73	8.4	0.564	87.70	0.064	84.73
7	9.5	0.657	87.50	0.072	84.40	9.4	0.645	87.50	0.073	84.40
8	10.5	0.708	87.30	0.081	84.06	10.4	0.697	87.30	0.082	84.06
9	11.8	0.840	87.05	0.091	83.73	11.6	0.811	87.05	0.091	83.73
10	13.5	0.947	86.72	0.104	83.40	13.0	0.909	86.72	0.102	83.40
11	13.9	0.969	86.26	0.106	82.98	13.7	0.940	86.26	0.107	82.96
12	14.4	0.991	85.82	0.110	82.57	14.4	0.979	85.82	0.112	82.52
13	14.9	1.011	85.39	0.114	82.17	15.1	1.003	85.40	0.117	82.10
14	15.5	1.037	84.97	0.118	81.77	15.8	1.031	84.98	0.122	81.68
15	16.1	1.057	84.58	0.122	81.39	16.5	1.048	84.59	0.127	81.28
16	16.7	1.077	84.19	0.126	81.00	17.3	1.079	84.20	0.133	80.88
17	17.3	1.095	83.82	0.131	80.63	18.1	1.099	83.82	0.138	80.49
18	18.0	1.112	83.46	0.135	80.26	18.9	1.118	83.47	0.142	80.11
19	18.7	1.131	83.12	0.139	79.90	19.8	1.140	83.13	0.148	79.73
20	19.5	1.150	82.80	0.144	79.55	20.7	1.159	82.82	0.154	79.47
21	20.3	1.169	82.49	0.149	79.21	21.6	1.177	82.51	0.160	79.02
22	21.1	1.184	82.19	0.154	78.87	22.5	1.195	82.21	0.165	78.67
23	22.0	1.202	81.91	0.159	78.54	23.4	1.208	81.93	0.170	78.33
24	22.9	1.219	81.64	0.165	78.22	24.4	1.226	81.66	0.176	78.00
25	23.9	1.237	81.39	0.169	77.90	25.4	1.241	81.41	0.182	77.67
26	24.9	1.252	81.15	0.175	77.59	26.5	1.251	81.17	0.187	77.36
27	25.9	1.266	80.93	0.179	77.29	27.5	1.269	80.95	0.193	77.06
28	27.0	1.282	80.72	0.186	77.00	28.6	1.282	80.74	0.198	76.76
29	28.1	1.297	80.53	0.193	76.71	29.7	1.297	80.55	0.204	76.47
30	29.2	1.311	80.35	0.198	76.43	30.9	1.310	80.37	0.210	76.19
31	30.4	1.324	80.19	0.204	76.16	32.0	1.322	80.21	0.216	75.92
32	31.6	1.338	80.04	0.210	75.90	33.2	1.334	80.07	0.221	75.66
33	32.8	1.351	79.91	0.215	75.64	34.4	1.346	79.94	0.227	75.40
34	34.1	1.363	79.79	0.221	75.39	35.7	1.358	79.82	0.233	75.16
35	35.4	1.375	79.69	0.227	75.15	37.0	1.369	79.72	0.239	74.92
36	36.8	1.389	79.60	0.233	74.91	38.3	1.380	79.63	0.245	74.69

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
37	38.1	1.399	79.52	0.239	74.68	39.6	1.391	79.55	0.250	74.47
38	39.6	1.411	79.46	0.245	74.46	40.9	1.400	79.49	0.255	74.26
39	41.0	1.423	79.42	0.251	74.25	42.3	1.411	79.45	0.261	74.06
40	42.5	1.434	79.39	0.257	74.04	43.7	1.422	79.42	0.267	73.86
41	44.1	1.446	79.36	0.264	73.95	45.1	1.432	79.39	0.272	73.78
42	45.7	1.457	79.34	0.269	73.87	46.6	1.441	79.37	0.278	73.72
43	47.3	1.468	79.32	0.276	73.74	48.1	1.451	79.35	0.284	73.60
44	48.9	1.478	79.30	0.281	73.62	49.6	1.460	79.33	0.289	73.50
45	50.6	1.488	79.28	0.288	73.50	51.1	1.468	79.31	0.294	73.39
46	52.3	1.498	79.26	0.293	73.39	52.7	1.478	79.29	0.300	73.30
47	54.1	1.507	79.24	0.299	73.28	54.3	1.487	79.27	0.306	73.21
48	55.9	1.518	79.22	0.305	73.17	55.9	1.495	79.25	0.311	73.12
49	57.7	1.527	79.21	0.311	73.07	57.5	1.503	79.24	0.316	72.05
50	59.6	1.537	79.19	0.317	72.97	59.2	1.512	79.23	0.322	72.97
51	61.5	1.546	79.17	0.323	72.88	60.9	1.520	79.21	0.327	72.88
52	63.4	1.555	79.15	0.329	72.79	62.6	1.528	79.19	0.332	72.79
53	65.4	1.563	79.14	0.334	72.69	64.3	1.535	79.18	0.338	72.69
54	67.4	1.572	79.12	0.340	72.60	66.1	1.543	79.16	0.343	72.60
55	69.5	1.581	79.10	0.346	72.51	67.9	1.551	79.14	0.348	72.51
56	71.6	1.589	79.08	0.352	72.43	69.7	1.558	79.12	0.353	72.43
57	73.7	1.597	79.07	0.358	72.35	71.6	1.565	79.11	0.359	72.35
58	75.9	1.606	79.05	0.363	72.27	73.4	1.573	79.09	0.364	72.27
59	78.1	1.614	79.04	0.369	72.19	75.3	1.580	79.08	0.370	72.19
60	80.3	1.622	79.02	0.375	72.11	77.3	1.587	79.06	0.375	72.11
61	82.5	1.629	79.00	0.380	72.04	79.2	1.594	79.04	0.380	72.04
62	84.9	1.637	78.99	0.386	71.97	81.2	1.601	79.02	0.385	71.97
63	87.2	1.644	78.97	0.391	71.91	83.2	1.607	79.01	0.389	71.91
64	89.6	1.652	78.96	0.397	71.84	85.2	1.614	78.99	0.394	71.84
65	92.0	1.659	78.94	0.402	71.77	87.3	1.621	78.98	0.399	71.77
66	94.5	1.666	78.93	0.407	71.71	89.4	1.627	78.97	0.404	71.72
67	97.0	1.673	78.92	0.413	71.65	91.5	1.633	78.96	0.409	71.66
68	99.5	1.681	78.90	0.418	71.60	93.6	1.639	78.94	0.414	71.61
69	102.1	1.688	78.89	0.424	71.54	95.8	1.645	78.93	0.419	71.54
70	104.7	1.695	78.88	0.429	71.48	98.0	1.651	78.92	0.424	71.50
71	107.3	1.702	78.87	0.434	71.43	100.2	1.657	78.91	0.429	71.45
72	110.0	1.709	78.85	0.439	71.38	102.4	1.663	78.89	0.433	71.41
73	112.7	1.715	78.84	0.445	71.32	104.7	1.669	78.88	0.438	71.36
74	115.5	1.722	78.82	0.450	71.27	107.0	1.675	78.86	0.442	71.32

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
75	118.3	1.729	78.81	0.455	71.22	109.3	1.681	78.85	0.447	71.27
76	121.1	1.735	78.80	0.460	71.18	111.6	1.687	78.84	0.451	71.23
77	124.0	1.741	78.79	0.465	71.13	114.0	1.692	78.83	0.456	71.19
78	126.8	1.746	78.77	0.470	71.09	116.4	1.698	78.82	0.460	71.15
79	129.8	1.752	78.76	0.475	71.04	118.8	1.703	78.81	0.465	71.11
80	132.8	1.758	78.75	0.480	71.00	121.3	1.709	78.80	0.469	71.07
81	134.7	1.762	78.74	0.483	70.96	122.6	1.712	78.79	0.471	71.03
82	136.5	1.765	78.73	0.486	70.92	124.0	1.715	78.78	0.474	71.00
83	138.4	1.769	78.72	0.488	70.89	125.4	1.717	78.77	0.476	70.96
84	140.2	1.772	78.71	0.491	70.85	126.8	1.720	78.76	0.479	70.93
85	142.0	1.776	78.70	0.494	70.81	128.1	1.723	78.75	0.481	70.89
86	143.7	1.779	78.69	0.497	70.78	129.5	1.726	78.74	0.483	70.86
87	145.5	1.782	78.68	0.499	70.74	130.8	1.728	78.73	0.485	70.83
88	147.2	1.785	78.67	0.502	70.71	132.1	1.731	78.72	0.488	70.80
89	148.9	1.788	78.66	0.504	70.67	133.4	1.733	78.71	0.490	70.77
90	150.5	1.791	78.65	0.507	70.64	134.6	1.736	78.70	0.492	70.74
91	152.1	1.794	78.64	0.509	70.61	135.8	1.738	78.69	0.494	70.72
92	153.7	1.797	78.63	0.511	70.58	137.1	1.740	78.68	0.496	70.69
93	155.3	1.799	78.62	0.514	70.56	138.3	1.743	78.67	0.497	70.67
94	156.9	1.802	78.61	0.516	70.53	139.4	1.745	78.66	0.499	70.64
95	158.4	1.805	78.60	0.518	70.50	140.6	1.747	78.65	0.501	70.62
96	160.0	1.807	78.59	0.520	70.48	141.8	1.749	78.64	0.503	70.60
97	161.4	1.810	78.58	0.522	70.45	142.9	1.751	78.63	0.505	70.58
98	162.9	1.812	78.57	0.525	70.43	144.0	1.752	78.62	0.506	70.55
99	164.3	1.815	78.56	0.527	70.40	145.1	1.754	78.61	0.508	70.53
100	165.8	1.817	78.55	0.529	70.38	146.2	1.756	78.60	0.510	70.51
101	167.2	1.819	78.54	0.531	70.36	147.3	1.758	78.59	0.512	70.49
102	168.6	1.821	78.53	0.533	70.34	148.3	1.760	78.58	0.514	70.47
103	170.0	1.824	78.53	0.534	70.32	149.4	1.762	78.58	0.515	70.46
104	171.3	1.826	78.52	0.536	70.30	150.4	1.764	78.57	0.517	70.44
105	172.7	1.828	78.51	0.538	70.28	151.4	1.766	78.56	0.519	70.42
106	174.0	1.830	78.50	0.540	70.26	152.4	1.768	78.55	0.520	60.41
107	175.3	1.832	78.49	0.541	70.25	153.4	1.770	78.54	0.522	70.40
108	176.6	1.833	78.48	0.543	70.23	154.4	1.772	78.53	0.523	70.38
109	177.9	1.835	78.47	0.544	70.22	155.3	1.774	78.52	0.525	70.37
110	179.1	1.837	78.46	0.546	70.20	156.3	1.775	78.51	0.526	70.36
111	180.4	1.839	78.45	0.547	70.19	157.2	1.776	78.50	0.527	70.35
112	181.6	1.841	78.44	0.549	70.17	158.2	1.778	78.49	0.528	70.34

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
113	182.8	1.842	78.44	0.550	70.15	159.1	1.779	78.49	0.530.	70.32
114	184.0	1.844	78.43	0.552	70.14	160.0	1.781	78.48	0.531	70.31
115	185.2	1.846	78.42	0.553	70.13	160.9	1.782	78.47	0.532	70.30
116	186.4	1.848	78.41	0.555	70.12.	161.8	1.783	78.46	0.533	70.29
117	187.5	1.849	78.40	0.556	70.11	162.6	1.785	78.46	0.535	70.28
118	188.7	1.851	78.40	0.558	70.09	163.5	1.786	78.45	0.536	70.27
119	189.7	1.852	78.39	0.559	70.08	164.3	1.788	78.45	0.538	70.26
120	190.9	1.854	78.38	0.561	70.07	165.2	1.789	78.44	0.539	70.25
121	192.0	1.855	78.37	0.562	70.06	166.0	1.790	78.43	0.540	70.25
122	193.1	1.857	78.37	0.563	70.06	166.8	1.791	78.43	0.541	70.24
123	194.1	1.858	78.36	0.564	70.05	167.6	1.793	78.42	0.542	70.24
124	195.2	1.860	78.36	0.565	70.05	168.4	1.794	78.42	0.543	70.23
125	196.2	1.861	78.35	0.566	70.04	169.2	1.795	78.41	0.544	70.23
126	197.3	1.862	78.34	0.567	70.03	170.0	1.796	78.40	0.545	70.23
127	198.3	1.863	78.33	0.569	70.03	170.7	1.798	78.39	0.546	70.23
128	199.3	1.865	78.33	0.570	70.02	171.5	1.799	78.39	0.546	70.22
129	200.3	1.866	78.32	0.572	70.02	172.3	1.801	78.38	0.547	70.22
130	201.2	1.867	78.31	0.573	70.01	173.0	1.802	78.37	0.548	70.22
131	202.2	1.868	78.30	0.574	70.01	173.7	1.803	78.36	0.549	70.22
132	203.2	1.870	78.30	0.575	70.01	174.5	1.804	78.36	0.550	70.22
133	204.1	1.871	78.29	0.576	70.00	175.2	1.804	78.35	0.551	70.22
134	205.1	1.873	78.29	0.577	70.00	175.9	1.805	78.35	0.552	70.22
135	206.0	1.874	78.28	0.578	70.00	176.2	1.806	78.34	0.553	70.22
136	206.9	1.875	78.27	0.579	70.00	176.5	1.807	78.33	0.554	70.22
137	207.8	1.876	78.26	0.580	70.00	176.9	1.808	78.32	0.555	70.22
138	208.7	1.877	78.26	0.580	70.00	177.6	1.809	78.32	0.555	70.22
139	209.6	1.878	78.25	0.581	70.00	178.3	1.810	78.31	0.556	70.22
140	210.5	1.879	78.24	0.582	70.00	179.9	1.811	78.30	0.557	70.22
141	211.3	1.880	78.24	0.583	70.00	180.6	1.812	78.30	0.558	70.22
142	212.2	1.881	78.23	0.584	70.00	181.2	1.813	78.29	0.559	70.22
143	213.0	1.882	78.23	0.584	70.00	181.8	1.813	78.29	0.559	70.22
144	213.9	1.883	78.22	0.585	70.00	182.5	1.814	78.28	0.560	70.22
145	214.7	1.884	78.22	0.586	70.00	183.1	1.815	78.28	0.561	70.22
146	215.5	1.885	78.21	0.587	70.00	183.7	1.816	78.27	0.562	70.22
147	216.3	1.886	78.21	0.588	70.00	184.3	1.817	78.27	0.562	70.22
148	217.1	1.887	78.20	0.588	70.00	184.9	1.817	78.26	0.563	70.22
149	217.9	1.887	78.20	0.589	70.00	185.5	1.818	78.26	0.564	70.22
150	218.7	1.888	78.19	0.590	70.00	186.1	1.819	78.25	0.565	70.22

HENRY H. DONALDSON

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
151	219.5	1.889	78.19	0.591	70.00	186.7	1.820	78.25	0.565	70.22
152	220.2	1.890	78.18	0.592	70.00	187.2	1.821	78.24	0.566	70.22
153	221.0	1.891	78.18	0.592	70.00	187.8	1.821	78.24	0.567	70.22
154	221.7	1.892	78.17	0.593	70.00	188.4	1.822	78.23	0.568	70.22
155	222.5	1.893	78.17	0.594	70.00	188.9	1.823	78.23	0.568	70.22
156	223.2	1.894	78.16	0.595	70.00	189.5	1.824	78.22	0.569	70.22
157	223.9	1.895	78.16	0.586	70.00	190.0	1.825	78.22	0.570	70.22
158	224.7	1.896	78.15	0.596	70.00	190.6	1.825	78.21	0.571	70.22
159	225.3	1.897	78.15	0.597	70.00	191.1	1.826	78.21	0.571	70.22
160	226.0	1.898	78.14	0.598	70.00	191.6	1.827	78.20	0.572	70.22
161	226.7	1.899	78.14	0.599	70.00	192.1	1.828	78.20	0.573	70.22
162	227.4	1.900	78.13	0.600	70.00	192.6	1.829	78.19	0.574	70.22
163	228.1	1.901	78.13	0.600	70.00	193.2	1.829	78.19	0.574	70.22
164	228.8	1.902	78.12	0.601	70.00	193.6	1.830	78.18	0.575	70.22
165	229.4	1.902	78.12	0.602	70.00	194.2	1.831	78.18	0.576	70.22
166	230.1	1.903	78.12	0.603	70.00	194.6	1.832	78.18	0.576	70.22
167	230.7	1.903	78.12	0.603	70.00	195.1	1.832	78.18	0.577	70.22
168	231.4	1.904	78.12	0.604	70.00	195.6	1.833	78.18	0.577	70.22
169	232.0	1.904	78.12	0.604	70.00	196.1	1.833	78.18	0.578	70.22
170	232.6	1.905	78.12	0.605	70.00	196.5	1.834	78.18	0.578	70.22
171	233.3	1.906	78.12	0.605	70.00	197.0	1.834	78.18	0.579	70.22
172	233.9	1.906	78.12	0.606	70.00	197.5	1.835	78.18	0.579	70.22
173	234.5	1.907	78.12	0.606	70.00	197.9	1.835	78.18	0.580	70.22
174	235.1	1.907	78.12	0.607	70.00	198.4	1.836	78.18	0.580	70.22
175	235.7	1.908	78.12	0.608	70.00	198.8	1.837	78.18	0.581	70.22
176	236.3	1.909	78.12	0.608	70.00	199.3	1.837	78.18	0.581	70.22
177	236.9	1.909	78.12	0.609	70.00	199.7	1.838	78.18	0.582	70.22
178	237.4	1.910	78.11	0.609	69.99	200.1	1.838	78.17	0.582	70.22
179	238.0	1.910	78.11	0.610	69.99	200.6	1.839	78.17	0.583	70.22
180	238.6	1.911	78.11	0.610	69.99	201.0	1.839	78.17	0.583	70.22
181	239.1	1.912	78.11	0.611	69.99	201.4	1.840	78.17	0.584	70.22
182	239.7	1.912	78.11	0.612	69.99	201.8	1.841	78.17	0.584	70.22
183	240.2	1.913	78.11	0.612	69.99	202.2	1.841	78.17	0.585	70.22
184	240.8	1.913	78.11	0.613	69.99	202.6	1.842	78.17	0.585	70.22
185	241.3	1.914	78.11	0.613	69.99	203.0	1.842	78.17	0.586	70.22
186	241.8	1.915	78.11	0.814	69.99	203.4	1.843	78.17	0.586	70.22
187	242.3	1.915	78.11	0.614	69.99	203.8	1.843	78.17	0.587	70.22
188	242.9	1.916	78.11	0.615	69.99	204.2	1.844	78.17	0.587	70.22

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
189	243.4	1.916	78.11	0.615	69.99	204.6	1.844	78.17	0.588	70.22
190	243.9	1.917	78.11	0.616	69.99	204.9	1.845	78.17	0.588	70.22
191	244.4	1.917	78.11	0.616	69.99	205.3	1.845	78.17	0.588	70.22
192	244.9	1.918	78.11	0.617	69.99	205.7	1.846	78.17	0.589	70.22
193	245.4	1.918	78.11	0.617	69.98	206.0	1.846	78.17	0.589	70.22
194	245.9	1.919	78.11	0.618	69.98	206.4	1.847	78.17	0.589	70.22
195	246.3	1.919	78.11	0.618	69.98	206.7	1.847	78.17	0.590	70.21
196	246.8	1.920	78.11	0.618	69.98	207.1	1.847	78.17	0.590	70.21
197	247.3	1.920	78.10	0.619	69.97	207.4	1.848	78.17	0.591	70.21
198	247.8	1.921	78.10	0.619	69.97	207.8	1.848	78.17	0.591	70.21
199	248.2	1.921	78.10	0.620	69.97	208.1	1.849	78.17	0.591	70.21
200	248.6	1.922	78.10	0.620	69.97	208.4	1.849	78.17	0.592	70.20
201	249.1	1.922	78.10	0.620	69.96	208.8	1.849	78.17	0.592	70.20
202	249.6	1.923	78.10	0.621	69.96	209.1	1.850	78.17	0.592	70.20
203	250.0	1.923	78.10	0.621	69.96	209.4	1.850	78.16	0.593	70.20
204	250.4	1.924	78.10	0.622	69.96	209.8	1.851	78.16	0.593	70.20
205	250.9	1.924	78.10	0.622	69.95	210.1	1.851	78.16	0.593	70.20
206	251.3	1.925	78.10	0.622	69.95	210.4	1.851	78.16	0.594	70.19
207	251.7	1.925	78.10	0.623	69.95	210.7	1.852	78.16	0.594	70.19
208	252.1	1.926	78.10	0.623	69.95	211.0	1.852	78.16	0.594	70.19
209	252.5	1.926	78.09	0.624	69.94	211.3	1.853	78.16	0.595	70.19
210	252.9	1.927	78.09	0.624	69.94	211.6	1.853	78.16	0.595	70.19
211	253.4	1.927	78.09	0.624	69.94	211.9	1.853	78.16	0.596	70.19
212	253.7	1.928	78.09	0.625	69.94	212.2	1.854	78.16	0.596	70.18
213	254.2	1.928	78.09	0.625	69.93	212.5	1.854	78.16	0.596	70.18
214	254.5	1.929	78.09	0.626	69.93	212.8	1.855	78.16	0.597	70.18
215	254.9	1.929	78.09	0.626	69.93	213.1	1.855	78.16	0.597	70.18
216	255.3	1.929	78.09	0.626	69.93	213.4	1.855	78.16	0.597	70.18
217	255.7	1.930	78.09	0.627	69.92	213.7	1.856	78.16	0.597	70.17
218	256.1	1.930	78.08	0.627	69.92	213.9	1.856	78.15	0.598	70.17
219	256.4	1.930	78.08	0.627	69.92	214.2	1.856	78.15	0.598	70.17
220	256.8	1.931	78.08	0.628	69.91	214.4	1.857	78.15	0.598	70.16
221	257.2	1.931	78.08	0.628	69.91	214.7	1.857	78.15	0.598	70.16
222	257.5	1.931	78.08	0.628	69.90	215.0	1.857	78.15	0.599	70.16
223	257.9	1.932	78.07	0.629	69.90	215.2	1.858	78.14	0.599	70.15
224	258.2	1.932	78.07	0.629	69.90	215.5	1.858	78.14	0.599	70.15
225	258.6	1.932	78.07	0.629	69.89	215.8	1.858	78.14	0.599	70.15
226	258.9	1.933	78.07	0.630	69.89	216.0	1.859	78.14	0.600	70.14

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
227	259.2	1.933	78.07	0.630	69.89	216.2	1.859	78.14	0.600	70.14
228	259.6	1.933	78.06	0.630	69.88	216.5	1.859	78.13	0.600	70.14
229	259.9	1.933	78.06	0.630	69.88	216.7	1.859	78.13	0.600	70.14
230	260.2	1.934	78.06	0.631	69.88	217.0	1.860	78.13	0.601	70.13
231	260.6	1.934	78.06	0.631	69.87	217.2	1.860	78.13	0.601	70.13
232	260.9	1.934	78.06	0.631	69.87	217.5	1.860	78.13	0.601	70.13
233	261.2	1.935	78.05	0.632	69.87	217.7	1.861	78.12	0.601	70.12
234	261.5	1.935	78.05	0.632	69.86	217.9	1.861	78.12	0.602	70.12
235	261.9	1.935	78.05	0.632	69.86	218.1	1.861	78.12	0.602	70.12
236	262.1	1.936	78.05	0.633	69.85	218.3	1.862	78.12	0.602	70.11
237	262.4	1.936	78.05	0.633	69.85	218.6	1.862	78.12	0.602	70.11
238	262.8	1.936	78.04	0.633	69.85	218.8	1.862	78.11	0.603	70.11
239	263.0	1.937	78.04	0.634	69.84	219.0	1.863	78.11	0.603	70.10
240	263.3	1.937	78.04	0.634	69.84	219.2	1.863	78.11	0.603	70.10
241	263.6	1.937	78.04	0.634	69.84	219.4	1.863	78.11	0.603	70.10
242	263.9	1.938	78.03	0.634	69.83	219.6	1.863	78.10	0.603	70.09
243	264.2	1.938	78.03	0.635	69.83	219.8	1.863	78.10	0.604	70.09
244	264.5	1.938	78.03	0.635	69.82	220.0	1.864	78.10	0.604	70.08
245	264.8	1.938	78.03	0.635	69.82	220.3	1.864	78.10	0.604	70.08
246	265.0	1.939	78.02	0.635	69.81	220.4	1.864	78.09	0.604	70.07
247	265.3	1.939	78.02	0.636	69.81	220.6	1.864	78.09	0.604	70.07
248	265.6	1.939	78.02	0.636	69.80	220.8	1.864	78.09	0.605	70.06
249	265.8	1.940	78.01	0.636	69.80	221.0	1.864	78.08	0.605	70.06
250	266.1	1.940	78.01	0.636	69.79	221.2	1.865	78.08	0.605	70.05
251	266.3	1.940	78.01	0.637	69.79	221.4	1.865	78.08	0.605	70.05
252	266.6	1.940	78.01	0.637	69.78	221.6	1.865	78.08	0.605	70.04
253	266.8	1.941	78.00	0.637	69.78	221.7	1.865	78.07	0.606	70.04
254	267.1	1.941	78.00	0.637	69.77	221.9	1.865	78.07	0.606	70.03
255	267.3	1.941	78.00	0.638	69.77	222.1	1.865	78.07	0.606	70.03
256	267.6	1.941	78.00	0.638	69.76	222.3	1.866	78.07	0.606	70.02
257	267.8	1.942	77.99	0.638	69.76	222.4	1.866	78.06	0.606	70.02
258	268.0	1.942	77.99	0.638	69.75	222.6	1.866	78.06	0.607	70.01
259	268.3	1.942	77.99	0.639	69.75	222.8	1.866	78.06	0.607	70.01
260	268.5	1.943	77.98	0.639	69.74	223.0	1.866	78.05	0.607	70.00
261	268.7	1.943	77.98	0.639	69.74	223.1	1.866	78.05	0.607	70.00
262	269.0	1.943	77.98	0.639	69.73	223.3	1.867	78.05	0.607	69.99
263	269.2	1.943	77.98	0.640	69.73	223.4	1.867	78.05	0.608	69.99
264	269.4	1.944	77.97	0.640	69.72	223.6	1.867	78.04	0.608	69.98

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
265	269.6	1.944	77.97	0.640	69.72	223.7	1.867	78.04	0.608	69.98
266	269.8	1.944	77.97	0.640	69.72	223.9	1.867	78.04	0.608	69.98
267	270.0	1.944	77.96	0.640	69.71	224.0	1.867	78.03	0.608	69.97
268	270.2	1.944	77.96	0.640	69.71	224.2	1.867	78.03	0.608	69.97
269	270.5	1.945	77.96	0.640	69.70	224.3	1.867	78.03	0.608	69.96
270	270.7	1.945	77.95	0.641	69.70	224.5	1.868	78.02	0.609	69.96
271	270.9	1.945	77.95	0.641	69.69	224.6	1.868	78.02	0.609	69.95
272	271.1	1.945	77.94	0.641	69.69	224.8	1.868	78.02	0.609	69.95
273	271.3	1.945	77.94	0.641	69.68	224.9	1.868	78.01	0.609	69.94
274	271.5	1.945	77.94	0.641	69.68	225.0	1.868	78.01	0.609	69.94
275	271.6	1.946	77.93	0.641	69.67	225.1	1.868	78.01	0.609	69.94
276	271.8	1.946	77.93	0.641	69.67	225.3	1.868	78.00	0.609	69.93
277	272.0	1.946	77.93	0.641	69.66	225.4	1.868	78.00	0.609	69.93
278	272.2	1.946	77.92	0.642	69.66	225.5	1.869	78.00	0.610	69.92
279	272.3	1.946	77.92	0.642	69.65	225.7	1.869	78.00	0.610	69.92
280	272.5	1.946	77.92	0.642	69.65	225.8	1.869	77.99	0.610	69.91
281	272.7	1.947	77.91	0.642	69.64	225.9	1.869	77.99	0.610	69.91
282	272.8	1.947	77.91	0.642	69.64	226.0	1.869	77.99	0.610	69.91
283	273.0	1.947	77.91	0.642	69.63	226.1	1.869	77.98	0.610	69.90
284	273.2	1.947	77.90	0.642	69.63	226.2	1.869	77.98	0.610	69.90
285	273.4	1.947	77.90	0.642	69.62	226.4	1.869	77.98	0.610	69.89
286	273.5	1.947	77.89	0.643	69.62	226.5	1.870	77.97	0.611	69.89
287	273.7	1.948	77.89	0.643	69.61	226.6	1.870	77.97	0.611	69.88
288	273.9	1.948	77.89	0.643	69.61	226.7	1.870	77.97	0.611	69.88
289	274.0	1.948	77.88	0.643	69.60	226.8	1.870	77.96	0.611	69.87
290	274.2	1.948	77.88	0.643	69.60	226.9	1.870	77.96	0.611	69.87
291	274.3	1.948	77.88	0.643	69.59	227.0	1.870	77.96	0.611	69.86
292	274.5	1.948	77.87	0.643	69.59	227.1	1.870	77.95	0.611	69.86
293	274.6	1.948	77.87	0.643	69.58	227.2	1.870	77.95	0.611	69.85
294	274.7	1.948	77.86	0.643	69.58	227.3	1.870	77.94	0.611	69.85
295	274.9	1.948	77.86	0.644	69.57	227.4	1.870	77.94	0.611	69.84
296	275.0	1.948	77.86	0.644	69.56	227.5	1.870	77.94	0.611	69.84
297	275.2	1.949	77.85	0.644	69.56	227.6	1.871	77.93	0.612	69.83
298	275.3	1.949	77.85	0.644	69.55	227.7	1.871	77.93	0.612	69.83
299	275.4	1.949	77.84	0.644	69.55	227.8	1.871	77.92	0.612	69.82
300	275.5	1.949	77.84	0.644	69.54	227.9	1.871	77.92	0.612	69.82
301	275.7	1.949	77.84	0.644	69.53	228.0	1.871	77.92	0.612	69.81
302	275.8	1.949	77.83	0.644	69.53	228.0	1.871	77.91	0.612	68.81

HENRY H. DONALDSON

TABLE 74—Continued

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
303	275.9	1.949	77.83	0.645	69.52	228.1	1.871	77.91	0.612	69.80
304	276.1	1.949	77.82	0.645	69.52	228.2	1.871	77.90	0.612	69.80
305	276.2	1.949	77.82	0.645	69.51	228.3	1.871	77.90	0.612	69.79
306	276.3	1.949	77.82	0.645	69.50	228.3	1.871	77.90	0.612	69.79
307	276.4	1.949	77.81	0.645	69.50	228.4	1.871	77.89	0.612	69.78
308	276.5	1.949	77.81	0.645	69.49	228.5	1.871	77.89	0.612	69.78
309	276.6	1.950	77.80	0.645	69.49	228.6	1.872	77.88	0.613	69.77
310	276.7	1.950	77.80	0.645	69.48	228.7	1.872	77.88	0.613	69.77
311	276.9	1.950	77.80	0.646	69.47	228.7	1.872	77.88	0.613	69.76
312	277.0	1.950	77.79	0.646	69.47	228.8	1.872	77.87	0.613	69.76
313	277.0	1.950	77.79	0.646	69.46	228.8	1.872	77.87	0.613	69.75
314	277.1	1.950	77.78	0.646	69.46	228.9	1.872	77.86	0.613	69.75
315	277.2	1.950	77.78	0.646	69.45	229.0	1.872	77.86	0.613	69.74
316	277.3	1.950	77.77	0.646	69.44	229.0	1.872	77.85	0.613	69.73
317	277.5	1.950	77.77	0.646	69.44	229.1	1.872	77.85	0.613	69.73
318	277.5	1.950	77.76	0.646	69.43	229.1	1.872	77.84	0.613	69.72
319	277.6	1.950	77.76	0.646	69.43	229.2	1.872	77.84	0.613	69.72
320	277.7	1.950	77.75	0.646	69.42	229.3	1.872	77.83	0.613	69.71
321	277.8	1.950	77.75	0.646	69.41	229.3	1.872	77.83	0.613	69.71
322	277.9	1.951	77.74	0.647	69.41	229.4	1.873	77.82	0.614	69.70
323	278.0	1.951	77.74	0.647	69.40	229.4	1.873	77.82	0.614	69.70
324	278.0	1.951	77.73	0.647	69.40	229.5	1.873	77.81	0.614	69.69
325	278.1	1.951	77.73	0.647	69.39	229.5	1.873	77.81	0.614	69.68
326	278.2	1.951	77.72	0.647	69.38	229.6	1.873	77.80	0.614	69.68
327	278.3	1.951	77.72	0.647	69.38	229.6	1.873	77.80	0.614	69.67
328	278.4	1.951	77.71	0.647	69.37	229.7	1.873	77.79	0.614	69.67
329	278.4	1.951	77.71	0.647	69.37	229.7	1.873	77.79	0.614	69.66
330	278.5	1.951	77.70	0.647	69.36	229.8	1.873	77.78	0.614	69.66
331	278.6	1.951	77.70	0.647	69.35	229.8	1.873	77.78	0.614	69.65
332	278.6	1.951	77.69	0.647	69.35	229.8	1.873	77.77	0.614	69.64
333	278.7	1.951	77.69	0.647	69.34	229.9	1.873	77.77	0.614	69.64
334	278.7	1.952	77.68	0.648	69.34	229.9	1.874	77.76	0.615	69.63
335	278.8	1.952	77.68	0.648	69.33	229.9	1.874	77.76	0.615	69.63
336	278.9	1.952	77.67	0.648	69.32	230.0	1.874	77.75	0.615	69.62
337	278.9	1.952	77.67	0.648	69.32	230.0	1.874	77.75	0.615	69.62
338	279.0	1.952	77.66	0.648	69.31	230.0	1.874	77.74	0.615	69.61
339	279.0	1.952	77.66	0.648	69.31	230.1	1.874	77.74	0.615	69.61
340	279.1	1.952	77.65	0.648	69.30	230.1	1.874	77.73	0.615	69.60

TABLE 74—Concluded

AGE IN DAYS	MALES					FEMALES				
	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord	Body weight gms.	Brain weight gms.	Per cent of water brain	Cord weight gms.	Per cent of water cord
341	279.2	1.952	77.64	0.648	69.29	230.1	1.874	77.72	0.615	69.59
342	279.2	1.952	77.64	0.648	69.29	230.1	1.874	77.72	0.615	69.59
343	279.3	1.952	77.63	0.648	69.28	230.2	1.874	77.71	0.615	69.58
344	279.3	1.952	77.63	0.648	69.27	230.2	1.874	77.71	0.615	69.57
345	279.3	1.952	77.62	0.648	69.27	230.2	1.874	77.70	0.615	69.57
346	279.4	1.952	77.61	0.648	69.26	230.3	1.874	77.69	0.615	69.56
347	279.4	1.953	77.61	0.648	69.25	230.3	1.874	77.69	0.615	69.56
348	279.5	1.953	77.60	0.648	69.25	230.3	1.874	77.68	0.615	69.55
349	279.5	1.953	77.60	0.648	69.24	230.3	1.874	77.68	0.615	69.54
350	279.6	1.953	77.59	0.648	69.23	230.3	1.874	77.67	0.615	69.54
351	279.6	1.953	67.58	0.648	69.23	230.3	1.874	77.66	0.615	69.53
352	279.6	1.953	77.58	0.648	69.22	230.3	1.874	77.66	0.615	69.52
353	279.7	1.953	77.57	0.649	69.21	230.4	1.875	77.65	0.616	69.52
354	279.7	1.953	77.57	0.649	69.20	230.4	1.875	77.65	0.616	69.51
355	279.7	1.953	77.56	0.649	69.20	230.4	1.875	77.64	0.616	69.50
356	279.8	1.953	77.55	0.649	69.19	230.4	1.875	77.63	0.616	69.50
357	279.8	1.953	77.55	0.649	69.18	230.4	1.875	77.63	0.616	69.49
358	279.8	1.953	77.54	0.649	69.18	230.4	1.875	77.62	0.616	69.48
359	279.8	1.954	77.54	0.649	69.17	230.4	1.875	77.62	0.616	69.48
360	279.8	1.954	77.53	0.649	69.16	230.4	1.875	77.61	0.616	69.47
361	279.8	1.954	77.52	0.649	69.16	230.4	1.875	77.60	0.616	69.47
362	279.9	1.954	77.52	0.649	69.15	230.4	1.875	77.60	0.616	69.46
363	279.9	1.954	77.51	0.649	69.14	230.4	1.875	77.59	0.616	69.45
364	279.9	1.954	77.51	0.649	69.14	230.4	1.875	77.59	0.616	69.45
365	279.9	1.954	77.50	0.649	69.13	230.4	1.875	77.58	0.616	69.44

SOME EXPERIMENTS ON THE NATURE AND FUNC-
TION OF REISSNER'S FIBER

GEORGE E. NICHOLLS

Beit Memorial Fellow

Zoological Department, King's College, London

CONTENTS

It is probable that concerning no part of the vertebrate ner-
vous system have there been held views more widely divergent
than those which have been entertained concerning Reissner's
fiber.

In 1907, when I took up the study of this structure, Sargent's
'optic reflex' theory had met with very general acceptance. At
an early stage in my work, however, I obtained proof that the

117

fiber, although undoubtedly a preformed structure, was certainly not a nerve fiber and, therefore, could not have the function ascribed to it by Sargent. In 1909, I published a statement to that effect and in the following summer, following my discovery of the practical accessibility of the fiber in the tail region, I carried out some experiments upon elasmobranchs, in an endeavour to ascertain the function of the fiber.

The results of these experiments, which were performed upon less than a dozen dogfish and rays, were hardly sufficient to give a conclusive answer to the question of the function of the fiber but were, nevertheless, extremely suggestive. An account of these experiments was published, therefore, in a short preliminary paper which did not, however, appear until 1912. In the meanwhile, a much more extensive series of experiments had been carried out but there had been no opportunity to examine this material microscopically before the paper in question was published.

The completion of the investigation has been very considerably delayed, for these further experiments were scarcely completed when I left England to take up an appointment in India. I had purposed however, to carry on, there, the work of preparing the necessary serial sections. The material is, unfortunately, exceedingly refractory, so that under the best of circumstances the preparation of serial sections demands much time and patience. In India, there were added difficulties, due to the climate, and the preparation of the sections went on very slowly, it being possible to attempt this work only during the cold weather. An attempt to get some of the material sectionized in England was unsuccessful, essential portions of some of the specimens being ruined in the attempt to prepare the sections and the remaining tails were returned to me as being too refractory to yield satisfactory sections by ordinary methods. In the end I was compelled to postpone the preparation of my remaining material for microscopic study until my return to England. Only recently has this part of the task been completed.

In the interval, I have published a paper ('12 a) dealing with the subject of Reissner's fiber and its relation to the central

nervous system. Accordingly, there will be need, at this time, only for a brief review of the various suggestions which have been put forward as to the nature and function of the fiber and a short account of the present state of our knowledge of the fiber and its connections, the reader being referred to the above mentioned work for further details.

I gladly avail myself of this opportunity to express my thanks to Professor Dendy for valuable advice and criticism throughout the progress of the work: also, to the Government Grant Committee of the Royal Society, for Grants in Aid; to the British Association for the Advancement of Science and the Senate of the University of London for placing at my disposal their tables at the Plymouth Marine Laboratory, and to Dr. Allen, Director of the Laboratory, for the facilities afforded me in the prosecution of the research.

I. INTRODUCTION

A. *A review of the suggestions which have been made concerning the nature and function of Reissner's fiber and the sub-commissural organ*

1. Reissner ('60), by whom the fiber which now bears his name was discovered, believed that this 'Centralfaden' was simply a nerve fiber and to him, therefore, it was remarkable principally on account of its peculiar situation. He found it, as is well known, lying freely as an axial thread in the central canal of the spinal cord of the lamprey. Since the diameter of the fiber in this animal (in which alone he had observed it) is, approximately, that of a moderately coarse nerve fiber, it is scarcely surprising that, its unusual situation notwithstanding, Reissner came to this conclusion. Kutschin ('63) who confirmed Reissner's discovery, accepted that author's view of its nature. Neither of these observers was able to trace the fiber into the brain ventricles and they believed it to be confined to the central canal of the spinal cord.

2. That a nerve fiber should occur in such a situation seemed to Stieda ('68, '73) altogether improbable and he decided that Reissner's fiber ('jenen räthselhaften Strang') must be an arti-

fact. He suggested that the alleged fiber was produced by the coagulation of the cerebro-spinal fluid under the action of the fixing reagent, pointing out that there was no evidence of its being related to any nerve cell.

For thirty years this view passed almost unquestioned, Viault ('76), Rohon ('77), Sanders ('78, '94) and Gadow ('91) all accepting it. More recently Kalberlah ('00), Streeter ('03) and Edinger ('08) have expressed themselves in agreement with Stieda's view. That this view was so widely held is, doubtless, the explanation of the fact that during this period there are found, in the literature, so few references to the occurrence of the fiber.

3. Interest in this structure revived, however, when Studnička ('99) reasserted the preformed nature of the fiber. This author suggested that it was to be regarded as an epithelial secretion, comparable to that which has produced the crystalline style of the lamellibranch gut. He believed that it is produced by the cells lining the central canal of the spinal cord and that it is capable of growing forward, to end freely in the brain ventricles but he made no suggestion as to its function. Kolmer ('05) appears to be the only author who has endorsed this view and Studnička has, himself, since abandoned it ('13).

4. It is a very surprising fact that the extraordinary and quite conspicuous development of the epithelium beneath the posterior commissure, should have remained for so long unnoticed. A brief mention of it, indeed, appears to have been made by Fulliquet ('86) but not until 1892 was it figured (very diagrammatically) by Edinger ('92) who conjectured that it might be a glandular body producing some secretion to be discharged into the cerebro-spinal fluid. Its histology was first carefully described by Studnička ('00) who gave figures of its finer anatomy in dogfish and lamprey but did not, apparently, realize its connection with Reissner's fiber.

5. A little later the sub-commissural organ of the Ammocoete was described and figured by Dendy ('02) who noted the existence of close-set cilia clothing its ventricular surface and suggested that, in conjunction with certain folds of the choroid plexus

of the midbrain, it served to establish currents which promoted the circulation of the encephalic fluid.

6. In the meanwhile Sargent had also asserted the preformed nature of Reissner's fiber but had denied that Studnička was correct in interpreting it as a secretion. In Sargent's view the fiber was a nervous structure.

In several subsequent papers ('01, '03, '04) Sargent endeavored to establish this view stating that Reissner's fiber consists of "numerous axis cylinders closely applied to each other and surrounded by a single thin medullary sheath of myelin." These axis cylinders were supposed to be derived in part from the numerous large cells of the 'Dachkern' and from alleged multipolar cells in the habenular ganglion as well as from other multipolar cells said to be situated actually within the lumen of the central canal, towards the hinder end of the spinal cord. In teleosts, in which group Sargent overlooked the remnants of the 'Dachkern,' he claimed that the alleged midbrain constituent "axons" of Reissner's fiber were derived from the myriad cells of the torus longitudinalis.

Reissner's fiber was, therefore, according to this author, built up of two sets of axons running in opposite directions and a comparison was made between this structure and the giant fibers of Amphioxus and Annelida. Concerning the destination of the forwardly running axons there is nothing stated, but those which were said to arise in the brain were regarded as motor axons having a very great length, each being supposed to stretch from the midbrain roof direct to one of the trunk muscles. Sargent stated that he had seen such fibers leaving the main Reissner's fiber in the region of the spinal cord and that these passed out directly to the musculature, probably by way of the ventral spinal roots. In the midbrain roof the related nerve cells were described as in direct connection with the proximal ending of the retinal neurons so that there was said to be interposed but a single nerve element between the sensory (retinal) nerve cell and the muscle-fiber in the trunk. Sargent suggested that, by this means, the delay in the transmission of motor stimuli along

the ordinary (tecto-spinal) conduction paths through a number of neurons could be lessened in cases of urgency.

Houser ('01) claimed that he had been able to confirm Sargent's observations, while numerous observers seem to have accepted Sargent's theory concerning the function of the fiber.

That, notwithstanding many weighty objections, this theory met with such general acceptance is doubtless to be attributed very largely to the fact that Sargent claimed ('04) that his observations had been fully confirmed by actual experiments upon living animals (vide infra).

7. Although Sargent ('03) was the first to describe the connection between Reissner's fiber and the sub-commissural organ (his 'ependymal groove') he attributed comparatively little importance to this latter structure, asserting that it served merely as a support and anchorage for Reissner's fiber. In this view he has been followed recently by Tretjakoff ('13).

Kölliker ('02) recording the occurrence of Reissner's fiber in the blind Proteus and other Amphibia, admitted that he had become convinced of the preformed nature of the fiber. He appears, however, to have been unable to choose between the conflicting views advanced by Studnička, Sargent and Kalberlah.

8. The work of Ayers upon 'Ventricular Fibers in Myxinoids' is of interest in that it contains the first suggestion that Reissner's fibers might be composed of numerous united delicate fibrillae springing from ependymal epithelial cells. Whether, however, he considers these fibrillae as of the same nature as the ependymal fibers which serve as supporting structures within the central nervous system, or not, Ayers does not make clear, and his work unfortunately contains a number of erroneous statements. He does not, indeed, refer to the fiber by name and appears to have been wholly unaware of previous work upon the subject.

Thus, in Bdellostoma, he figures numerous more or less parallel ventricular fibers which, while they may perhaps represent several lengths of a much folded and snarled fiber, may equally well represent some artifact. It certainly is not the normal condition in this animal. Moreover, it would appear that

Ayers never saw Reissner's fiber in the lamprey, since his description of the 'ventricular fibers' in that animal as "a fine-meshed network of fibrils which . . . . in life practically fills the ventricular cavity" certainly can not apply to Reissner's fibers. It is extremely probable, therefore, that Ayers failed to distinguish clearly between coagulum and the fibrillae of Reissner's fiber. His conclusion that the fiber was certainly "an organ of relation bringing *all parts* of the ventricular cavity into intimate connection" (my italics) is likewise mistaken, for Ayers did not correctly identify the brain cavities in this animal, in which of the iter little remains but the sub-commissural canal. Accordingly he failed to recognize the distinction which exists between the tract of modified epithelium which constitutes the sub-commissural organ and the flattened epithelium which lines other parts of the ventricular cavity. Concerning the function of the fiber he conjectured that it might be "connected with the control of the ventricular lymph supply by vaso-motor control."

9. Horsley ('08) describing the occurrence of Reissner's fiber in certain apes stated that, in these forms at least, the fiber had not the structure of a tract of nerve fibers nor, when cut, did it exhibit Wallerian degeneration. While not denying the accuracy of Sargent's statements in so far as they relate to this structure in the lower vertebrates, Horsley expressed the opinion that, in its resiliency, the fiber resembled a chitinous or skeletal structure and suggested that, in the higher vertebrates, it had become nothing more, perhaps, than a residual structure.

10. In 1909 I gave an account ('09) of the behavior of the fiber in recoil and stated that, in my opinion, the fiber was certainly non-nervous. At the same time Dendy ('09) put forward an entirely novel suggestion concerning the function of the fiber. His suggestion was that the fiber itself was a strand of connective tissue which played a merely mechanical part, variations in its tension being produced by the flexure of the body and every such variation might be supposed to result in a stimulus being transmitted to the cells of the sub-commissural organ This latter structure was interpreted as a sensory

organ, controlling automatically the flexure of the body. He concluded by expressing the hope that some way would be found of overcoming the apparently insuperable obstacles which stood in the way of satisfactory experiments upon the fiber by which alone could the hypothesis be tested.

11. A study of the development of Reissner's fiber in Cyclostomes (and Amphibia) led me to the conclusion ('12, '12a, '13) that this structure was formed by the coalescence of cilia-like processes springing from cells which, while largely collected upon the sub-commissural organ, are not limited to that organ, other cells occurring scattered in the ependymal lining of the central canal contributing to the fiber. In my opinion, the fiber is to be regarded as a thread of protoplasm. This view is supported by the staining reactions of the fiber, while its high refractivity, its power of regeneration and the rapidity with which it apparently disintegrates after death are facts easily explicable upon this hypothesis. Further, its mode of contraction is paralleled, only, so far as I am aware, in the scarcely modified protoplasm which forms the stalk of certain Protozoa.

This view that the fiber is, in fact, a protoplasmic thread has since been accepted by Dendy ('12), Studnička ('13) and by Tretjakoff ('13). The latter author, however, appears to have misread Sargent's papers, for he attributes this view to that investigator, saying ('13, p. 110) "Sargent zeigte nämlich, dass der Faden noch in embryonalen oder larvalen Leben als ein Bundel von feinen, cilienahnlichen Fortsätzen der Zellen der Sub-kommissuralen Grube ensteht."

12. Tretjakoff ('13), however, while accepting this view of the nature and function of the fiber suggests that we are mistaken ("ich glaube deswegen, dass in diesem Punkt die Theorie von Dendy und Nicholls falsch ist") in attributing any sensory function to the sub-commissural organ. He believes that the sensory cells connected with Reissner's fiber are found only in the epithelium which lines the central canal and holds, with Sargent, that the sub-commissural organ serves merely for the support or anchorage of the fiber. These sensory cells are

described by Tretjakoff as projecting into the lumen of the
central canal where each is said to end in a small knobbed proc-
ess, which Tretjakoff compares to the bellpush of an electric
bell.   He supposes that the stimulation of these cells is effected
by the pressure of the fiber upon these processes whenever the
body is flexed.

That Tretjakoff's investigations were made upon material
in which the fiber had been broken and had retracted is suggested
by his figures.   Two only of these depict the central canal.   In
one (fig. 20) the fiber (which is invariably very fine in the Ammo-
coete) is seen indistinct and vastly swollen.   In the other (fig.
19) the fiber is absent and the lumen of the central canal is
occupied by nuclear bodies, the remains probably of epithelial
cells dislodged from the ependymal epithelium by the fiber in
its withdrawal.   Under these circumstances it is not surprising
that Tretjakoff failed to find the delicate filaments which seem
to join the fiber at frequent intervals as I have described ('12 a)
and the occurrence of which has been confirmed by Studinčka
('13, p. 585).

The little knobs (Tretjakoff's bell-pushes) are almost certainly
the retracted remnants of the fibrillae of those cells which, in
my view contribute to the formation of the fiber and which,
torn free by the dislocation of the fiber, have shrunk back upon
the sensory process of the parent cell.

B. Earlier attempts to determine the function of Reissner's fiber by experimental methods

The first reference to experiment in connection with the ques-
tion of the function of Reissner's fiber occurs in a preliminary
paper by Sargent ('01).   These experiments were subsequently
described in greater detail in 1904.

In these experiments an attempt was made to break the fiber
by a means of incision made through the choroid plexus of the
fourth ventricle of certain elasmobranchs.   Such experiments,
involving, as they necessarily did, the risk of serious disturbance
to the central nervous system or even actual injury to the brain

itself, were of little value, for it could not be established that any of the reactions observed were the results simply of the interruption of the 'optic reflex short-circuit' alleged to be provided by Reissner's fiber.

I gather, moreover, that Sargent relied upon observations made from dissections to determine whether or not the experimental incision had really broken the fiber, which appears to me as an altogether unsatisfactory method. Whether there was a subsequent microscopical examination of the material is not clear nor does Sargent state what precautions were taken to prevent a disturbance of the fiber during the dissection. The statement that "the cord and medulla of each individual was preserved for microscopical examination" suggests that a part only of the nervous system was subsequently cut out. If this were the case, it is practically certain that, whatever the result of the experiment upon the fiber, it would be found retracted in the preserved material.

It is, therefore, a little difficult to ascertain the grounds for his remark ('01, p. 450) that "animals on which the equivalent operation was performed without breaking the fiber are nearly or quite normal."

Other experiments were made by Sargent ('01) to determine the effect of artificial extirpation of the eye upon the fiber but the results obtained were never recorded. Several years later, experiments were made upon Reissner's fiber by Horsley ('08). In this case the subjects of the experiments were individuals of two species of Macacus. Minute electrolytic lesions were made in the spinal cord, at the level of the fifth cervical segment, in order to break the fiber. No observations are recorded, however, upon the behavior of the living animals nor are details given as to the duration of the experiments. Concerning the appearance of the fiber under the microscope, Horsley remarked that Wallerian degeneration was not observed in the broken fiber.

I find, however, some little difficulty in interpreting the appearance of Reissner's fiber in the sections figured by Horsley.

In his figure 10, Reissner's fiber is seen in transverse section, occupying quite an appreciable part of the lumen of the central canal. As it is traced backwards from this level (the first cervical segment) through the third cervical segment (fig. 9) towards the point of lesion in the fifth cervical segment (fig. 8), it is seen to constantly diminish in size. Behind the point of lesion this diminution in size continues as will be seen in figures 11 and 12 but, more caudally, the diameter of the fiber is again seen to increase (fig. 13), this latter figure representing a section through the spinal cord in the lumbar region.

Now this is not at all what one would expect to find where Reissner's fiber had been broken experimentally. Usually it would be found that on either side of the lesion there was a stretch of canal devoid of fiber. Still further from the lesion the severed ends of the fiber might be found swollen and perhaps knotted if the material were killed and fixed soon after the lesion had been made. Tracing the fiber distally, in either direction, from these knotted or swollen ends one would expect to find that the fiber diminishes in diameter until the normal size is reached. If, however, the killing of the material were postponed for a considerable time after the experimental operation the swollen end and the spiral twisting would have disappeared and the fiber would have straightened out backwards, extending practically to the point of lesion, nearly normal except that it might not have regained its taut condition. The piece lying posterior to the lesion might have retracted wholly backwards to the end of the cord. If the material were not killed until several weeks after the operation it is probable that regeneration would have largely re-established the normal condition throughout.

The condition figured by Horsley, in which the fiber is most swollen anteriorly, regularly diminishes in diameter towards and past the lesion (and probably becomes normal in the thoracic region) but shows a renewed swelling very far back, suggests that the condition of the fiber may have had nothing to do with the actual experiment. I should judge that sufficient time had elapsed after the experiment to permit of regeneration, and the

actual condition of the fiber figured was due to its rupture in
removing the central nervous system from the body for pres-
ervation.   The appearances are those which would be observed
if the fiber were broken accidentally in the hindbrain and in the
region of the filum terminale by section of the nervous system
in those regions, or by handling during dissection.

From the figures it would appear that there may have been a
somewhat considerable local disturbance of the central nervous
system in consequence of the experiment.   While this might
have obscured, to some extent, the reaction consequent upon the
breaking of the fiber (especially as a point very far forward was
selected for the operation) it is nevertheless much to be regretted
that nothing is recorded as to the behavior of the living animals
as the result of the experiments.

C. An account of the present state of our knowledge of Reissner's fiber

Reissner's fiber is an extremely delicate protoplasmic thread,
having, in general, a diameter of more than 1μ and less than 3μ.
It possesses a high refractivity and, in the normal tense con-
dition, appears to be absolutely structureless.

It is normally present in the central nervous system of practi-
cally all vertebrates and may be seen, most readily, in longi-
tudinal (sagittal) sections of the spinal cord.   It is necessary,
however, that the nervous system shall have been preserved
entire and immediately after the death of the animal; even then,
carelessness in handling during the dissection may cause the
fiber to snap, or it may chance that the fiber was broken prior
to the death of the animal.   Generally, however, if the central
nervous system has escaped damage, Reissner's fiber will be
found everywhere in the central canal stretched taut and lying
centrally in the canal.   It maintains a uniform thickness and
shows no trace of spiral winding.   At frequent intervals it
appears to be connected with the ependymal epithelial cells by
delicate cilia-like protoplasmic filaments (figs. 29, 30).

In this undisturbed state the fiber issues (in the lower vertebrates at least) from the posterior end of the central canal through a terminal opening (the 'terminal neural pore') into the perineural space, where it ends in an elongated conical expansion (the 'terminal plug'). There is, in these forms, a widening of the lumen of the central canal at its posterior end to form a chamber (the 'terminal sinus') which is only incompletely enclosed by the walls of the filum terminale, this being, in this region, reduced to a simple epithelial tube. The posterior wall of the terminal sinus is formed by the meningeal sheath into which the terminal plug is inserted (text-figs. 1, 3).
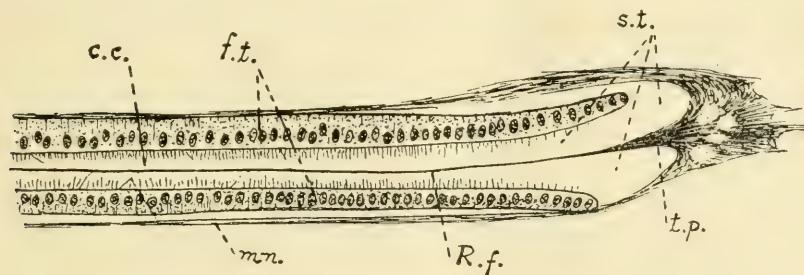
Text-fig. 1 Slightly diagrammatic median sagittal section through the end of the filum terminale to show the normal (undisturbed) arrangement of the sinus terminalis and the insertion of Reissner's fiber. c.c., central canal of the spinal cord (and terminal filament); f.t., filum terminale; mn., meninges, forming the hinder wall of the sinus terminalis; R.f., Reissner's fiber; s.t., sinus terminalis; t.p., terminal plug.

Traced forward, the normal fiber is found to pass from the central canal of the spinal cord into the fourth ventricle. It maintains its position in the middle line and appears, in this part of its course, to lie absolutely freely at the level of the middle of the height of the ventricle.

At the anterior part of the hindbrain, however, the fiber stretches in contact with the lower surface of the cerebellum. There is frequently, upon the lower surface of the rhombomesencephalic fold, a narrow groove (the 'isthmic canal') which may show traces of a paired character and which serves for the

reception of the fiber. Emerging from the anterior end of this groove, sometimes as a paired structure ('12 a, figs. 10, 11), Reissner's fiber stretches freely through the midbrain ventricle to the neighborhood of the posterior commissure.

The ventricular surface of the posterior commissure is clothed by a band of highly developed epithelium which is often folded in both the longitudinal and the transverse planes. It is to this remarkable tract of epithelium that the name 'sub-commissural organ' has been given. Owing to its longitudinal folding it has usually, in transverse sections, a horseshoe shape and partly encloses a median dorsal groove (the 'sub-commissural canal'). Reissner's fiber, if it has continued as an unpaired structure so far forward, breaks up at the hinder end of the posterior commissure into two or more strands which subdivide within this median groove into numerous delicate fibrillae which are connected with the cells of the sub-commissural organ.

A study of the development of the fiber indicates that it arises by the confluence of numerous filaments springing from sub-commissural organ and that the composite thread so formed extends backwards into the central canal of the spinal cord. Within the central canal it probably receives numerous additional components from scattered cells in the epithelium which lines the central canal.

Perhaps the most remarkable characteristic of the fiber is its extreme elasticity. In life it appears to exist under quite considerable tension and to be somewhat prone to accidental breakage. In that event, or following artificial section, the free ends may recoil sharply to form tangled knots or 'snarls.' The retraction is accompanied by a marked increase in the diameter of the fiber.

This elasticity usually disappears very rapidly during the process of fixation and the preserved fiber may become distinctly brittle (fig. 21). If, however, the fiber be severed before fixation is completed a retraction will still take place, but much more gradually, and it will then be found that the fiber has become wound in a more or less open spiral. Even where the recoil has been an abrupt one, resulting in the formation of the

characteristic knot, a careful examination of this mass will, almost invariably, reveal the fact that the retraction was accomplished by a spiral winding of the fiber.

Such a knot of retracted fiber has, indeed, the form of a contorted mass similar to that which may be produced in any thin stretched elastic thread of which one end is held fast and the other end twisted continuously in one direction. I have been able to obtain practically all stages intermediate between such complicated knots and the simplest spiral (text-fig. 2). Unlike
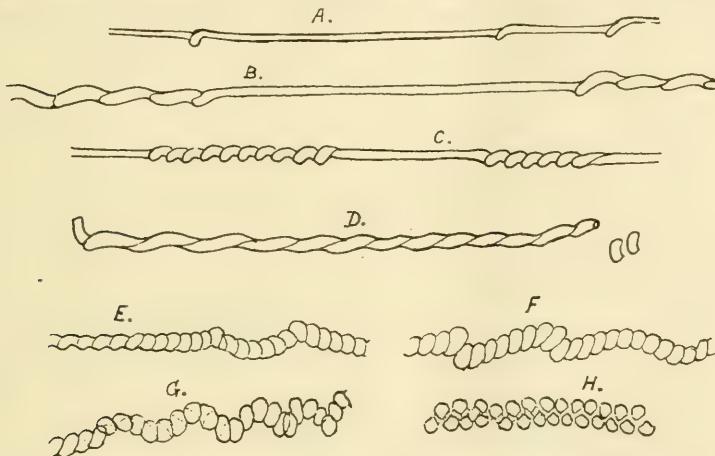
Text-fig. 2 Stages in the twisting of Reissner's fiber in its withdrawal from the point of breakage. *A, B, D* from Scyllium canicula (9); *C*, from Petromyzon fluviatilis; *E, F, G, H,* from Raia blanda (3).

the simple twisted elastic thread, however, the spiral winding may appear interruptedly in Reissner's fiber, spiral stretches alternating with swollen but untwisted lengths. Moreover, the twisting does not always make its appearance at the free end but may arise at a greater or less distance from the point where the fiber has been broken.

If, therefore, the spinal cord has been cut prior to fixation, Reissner's fiber may be found to have withdrawn for a relatively considerable distance from the point of section and a great stretch of the central canal may be found devoid of fiber. The extent of such retraction apparently varies with the region in which the

fiber has been broken and depends, possibly, upon the size of the
central canal in that particular region for, in the case of a sud-
den recoil, the spiral winding may produce at or near the severed
end a mass of coiled fiber which apparently checks further re-
traction.   With the retreating end of the fiber may be dragged
numerous epithelial cells and, around it, will collect a quantity
of coagulum (fig. 17) which may render it difficult to distinguish
exactly the condition of the knotted end.

On the side of the tangle remote from the point of section, the
fiber usually emerges as a coiled thread and thence passes gradu-
ally into a more open spiral.   If the fiber has been cut at a
sufficient distance from its attachment, this open spiral may pass
into a swollen but straight stretch and ultimately be found to
pass almost or quite into the normal condition.   Broken, how-
ever, near to one of its attachments, the fiber will almost cer-
tainly withdraw violently and completely to that attachment,
from which it may even tear itself free, dragging with it many
of the epithelial cells.

While this retraction which is so characteristic of Reissner's
fiber is, as I have pointed out ('09), altogether unlike anything
known in a nerve, neither does it altogether resemble the recoil
of a simple (homogeneous) elastic thread.   It is, therefore, of
especial interest that I have been able, recently, to detect in a
greatly swollen and retracted fiber what appears to be a fine
deeply staining central axis (fig. 17); the resemblance of the fiber
to the stalk of a Vorticella (with which I have already compared
it, '12 a, p. 25) is thereby greatly enhanced.   This appearance
is somewhat inconstant and never to be made out in the unrelaxed
condition.

I have been unable to decide whether the numerous delicate
fibrillae (fig. 29) seen in the central canal of the spinal cord are
in organic continuity with Reissner's fiber or whether they are
merely unusually long cilia which have been cemented to the
fiber after death by the coagulated cerebro-spinal fluid.   That
the former view is probably correct is indicated, I believe, by
the fact that in the cases in which some retraction of the fiber
has occurred it is very rare to find any of these fibrillae apparently

related to the swollen and retracted portion of the fiber. In-
stead, in the region in which there has been a dislocation of the
fiber, minute spherules of some highly refracting substance are
found plentifully, close to or in contact with the free surface of
the ependymal cells. That these are the contracted remains of
such connecting fibrillae, which were, indeed, component fila-
ments of Reissner's fiber is therefore extremely probable. The
withdrawal of the fiber would inevitably snap such connecting
filaments in the region affected and these broken protoplasmic
strands would naturally shrink backwards towards the surface
of the parent cells.

The view that Reissner's fiber is a thread of modified proto-
plasm, formed by the complete coalescence of numerous delicate
filaments (or hypertrophied cilia) is indicated by its origin and
is confirmed by its staining reactions. Moreover, it is an inter-
pretation which renders comprehensible its singular elastic recoil
notwithstanding its apparent structureless condition. Such spiral
retraction is met with only, so far as I am aware, in the little
differentiated protoplasm of the Protozoa, among which group
the fusion of cilia is also no uncommon feature.

II. THE SCOPE OF THE PRESENT INVESTIGATION

From what has been stated above it will be seen that, while
there has been a great variety in the suggestions made as to the
nature of Reissner's fiber, there have been put forward but three
theories as to its function.

The disproof of Sargent's statements as to the nature of the
fiber disposed, at the same time, of his 'optic reflex theory.'

Ayer's suggestion was based, as I have shown, almost entirely
upon an erroneous idea of the nature and normal condition of
the fiber and its relation to the ventricles; in any case his view is
not one which could easily be tested experimentally.

There remained Dendy's theory which might readily be put
to the test of experiment if a way could be devised of breaking
the fiber without damage to the central nervous system.

Such an operation became possible with my discovery ('10,
p. 527) of the actual condition of the hinder end of the filum

terminale in the Ichthyopsida. Elsewhere completely enveloped
by the brain and spinal cord, Reissner's fiber is peculiarly
accessible at the extremity of the tail, the more so that there is
practically an absence of nervous tissue in the hinder part of
the filum terminale. This structure is, indeed, little more than
a simple tube of columnar epithelium. At the actual hinder
end, Reissner's fiber may be said to be protected only by the
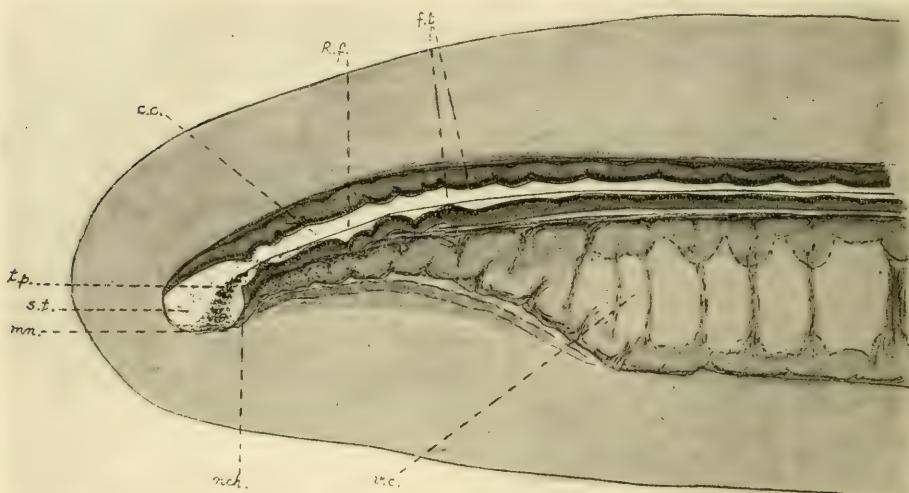
Text-fig. 3 A sagittal section through the extremity of the tail of Raia blanda
(III—experiment 3) to show the position of the sinus terminalis. *c.c.*, cen-
tral canal of the spinal cord (and terminal filament); *f.t.*, filum terminale; *mn.*,
meninges, forming the hinder wall of the sinus terminalis; *nch.*, notochord; *R.f.*,
Reissner's fiber; *s.t.*, sinus terminalis; *t.p.*, terminal plug; *v.c.*, vertebral column.

skin and the delicate meninges, between which there lies but a
film of connective tissue (text-fig. 3).

A cut made in the vicinity of the end of the terminal filament
would break the continuity of the fiber, therefore, but would be
quite unlikely to produce physiological results such as to mask or
interfere with the reactions resulting from the disorganization
of the mechanism of which Reissner's fiber forms a part.

My experiments, then, were intended primarily as an attempt
to determine the function of Reissner's fiber and its related

structures by means of observations made upon the living animals in which the continuity of the fiber had been intentionally destroyed, but I had other objects, also, in view.

At the time when the experiments were undertaken practically nothing was known concerning the mode of recoil of the fiber. Sargent had stated that when cut before fixation the free ends of the fiber retracted into a knotted mass or 'snarl' but he had not observed that this snarl was spirally wound. I had myself seen such snarls in several cases in material which had not been specially preserved for the study of this structure and in which the spinal cord had been cut previous to fixation ('12 a, figs. 17, 18, 19). In most of such material the fiber was ill preserved and, in the main, my own attention had been confined to a determination of the normal anatomical relations of the fiber. Accordingly, I had taken special precautions to thoroughly fix and harden my material before severing the spinal cord. Nevertheless, I had come, in the previous year, upon a few examples of this spirally wound condition which had been obtained unintentionally by a premature cutting of the spinal cord ('12 a, figs. 12, 16). These accidents, however, had yielded no information concerning the behavior of the fiber cut in life. It was naturally supposed that a breaking of the fiber in the living animal would be followed by a sharp recoil of the severed ends similar to that which was known to occur when the fiber was cut in freshly killed material. It was desirable, however, to ascertain if this were so.

It was anticipated, moreover, that the results of the experiments would throw light upon the question of the natural limits of this recoil. It must be remembered that beyond the mere fact of the occurrence of a recoil nothing had been recorded, and it was not even known whether the recoil started by the section of the living fiber would continue until both free ends had retracted to their respective points of attachment or whether, on the contrary, there would be formed speedily, in the living animal, a tangle (or tangles) which might (on reaching a size sufficient to block the lumen of the canalis centralis) automatically check further recoil in one or both directions.

In the latter event the tangled end (or ends) might perhaps afford a temporary hold and so prevent the fiber from being put completely out of action.

And, finally, there was the problem of regeneration. It was uncertain whether a tangle, if it were formed, would remain as a permanent record of the breaking of the fiber, or, if it were a transient feature, whether it would simply uncoil or whether the whole fiber, or the tangled part of it, simply disappeared to be replaced by a new growth.

Upon some of these points a certain amount of light was shed by the results of the few preliminary experiments carried out in 1910 but upon others the information was too meager to supply a decisive answer. Upon many of these points much additional knowledge has been gained from the more extended investigation carried out in the following year.

III. MATERIAL AND METHODS

The curiously exposed condition of the filum terminale in fishes, coupled with the fact that in both elasmobranchs and teleosts, Reissner's fiber is particularly well developed, largely influenced my choice of material. My final preference for elas-mobranchs was determined by the idea that the absence of bony tissue in the vertebral column would facilitate the preparation of the inevitable large number of series of sections.

I planned, originally, to experiment principally upon the common dogfish (Scyllium canicula) and to use rays only in the event of dogfish of suitable size being unobtainable. Knowing nothing certainly as to the probable extent of the recoil of the fiber, I was anxious to make use of comparatively small specimens, for it was possible that serial sections of the entire length of the central nervous system of all of the specimens might have to be prepared—a task of no little magnitude.

As it happened, only a couple of reasonably small dogfish were obtained during my stay in Plymouth in July, 1910; relatively small rays were, however, moderately plentiful, and for the most part the preliminary experiments were performed upon these animals.

Subsequent examination of this material under the microscope indicated that in most cases it would be necessary to examine only an inch or so of the spinal cord in front of the place where the incision was made. This point was almost always within a third of an inch of the extremity of the tail. The size of the specimen thus appeared to be of no great importance but this fact was only ascertained when the material had been prepared for microscopic examination nearly a year subsequently to the completion of these preliminary experiments.

Accordingly in the summer (August) of 1911 I was less careful to restrict my experiments to specimens of small size. I was thus enabled to obtain, more readily, the many specimens which I required. In all, a dozen comparatively small dogfish, ranging from 14 to 20 inches in length, were secured, and, upon these were performed experiments varying in duration from a few (three) hours in some cases to more than eighteen days in others. Of the rays, three species were employed, but of one of these, Raia microcellata, I had but a single specimen and, as in the previous year, the greater number of the experiments were made upon specimens of R. clavata and R. blanda. These included rays which were barely 6 inches in length and which were, presumably, just escaped from the egg case, while others ranged up to 16 inches. The duration of the experiments, in the case of the rays varied from a few (ten) minutes to as much as thirteen days.

The actual operation consisted in severing Reissner's fiber at a point quite near to the hinder end of the terminal filament and was practically nothing but a simple prick which rarely drew a drop of blood although, in some cases, the sections showed that there had been some effusion of blood into the canalis centralis.

Notwithstanding its trivial character, however, I was obliged by the conditions under which the vivisection license was issued, to perform the operation only upon anaesthetized specimens.

Some trial experiments with the anaesthetic indicated that dogfish were curiously susceptible to chloroform and, despite

my precautions, two of the subjects of the experiments subsequently failed to recover from the anaesthetic.

Finally it was found that a short immersion of the specimen in sea-water in which had been shaken up a small quantity of a mixture of chloroform and ether would induce a sufficient degree of insensibility, and this method was adopted throughout my series of experiments in 1911. Under this treatment, none of the specimens died.

The operation was quite easily performed, the subject being removed from the chloroform water and placed upon the table with its tail turned upon the side. The necessary prick was inflicted with the point of a very fine scalpel (which had previously been sterilized by passing through a gas flame) at a point usually considerably less than a third of an inch in front of the sinus terminalis. In the dogfish, therefore, the incision perforated the caudal fin near its hinder border while in the rays the cut was generally made behind the last dorsal fin (figs. 2, 8). The animal was at once returned to its tank, having been out of water for, perhaps, thirty seconds. Recovery was usually rapid and, as might be expected, there was no evidence of shock.

None of the specimens died from the effect of the operation, nor in the subsequent examination of the tissues in serial sections, was there found any indication that morbid or septic conditions had been set up. Indeed, apart from certain peculiarities of behavior about to be described, and which I attribute to the breaking of Reissner's fiber, the animals suffered no apparent ill-effects.

Nevertheless, two or three specimens were lost during the progress of the experiments from causes indirectly connected with the experiments. In the second series of experiments a number of photographs were taken, of normal specimens as well as of the subjects of the experiments. I could find no record of previous attempts to photograph living fish, and had accordingly to make a number of trial exposures. At first, attempts were made to obtain the photographs out of doors by daylight. Numerous difficulties cropped up however, for none of the out-

side tanks were glass fronted, and the only available glass-fronted tank, of a size to be readily transported, held but a comparatively small quantity of water and there were no facilities for connecting this tank with the aerating apparatus. A prolonged sojourn of the fish in this tank was not possible so that attempts to photograph under these conditions involved disturbing the specimens, transferring them in a bucket to the small tank and then waiting for them not only to settle down but to settle in a position in which it would be possible to photograph them. One or two lucky snapshots were obtained but the method was, in general, a failure.

An attempt to photograph the fish in their proper tanks in the laboratory encountered other difficulties. Of these the chief was connected with the light. With subdued daylight a comparatively long exposure was needed and it was found in practice that the head region was always blurred by the respiratory movements even if the fish did not elect to move bodily during the process.

In the end flash-light photographs were taken. The camera was fixed up opposite the tank in which was the specimen of which a photograph was desired and by the light of an incandescent gas lamp it was focussed upon a part of the tank a little within the glass front. Above the camera was stretched a piece of string upon which were placed a number of bent strips of magnesium ribbon. Usually some twenty inches of the ribbon were required, divided into four or more pieces. The gas lamp was then extinguished, and the aerating tube and bulb removed from the tank to do away with movement in the water. As soon as the specimen settled in a suitable position the strips of magnesium were lit, as nearly as possible, simultaneously. The reflection from the glass front of the tank was considerable, but in some of the later photographs this was diminished by igniting other strips of magnesium suspended immediately above the tank, care being taken to shield the lens from the direct rays from this source of illumination. Most of the photographs reproduced here were taken in this way.

In all, experiments were performed upon sixty-seven elasmo-branchs, of which twelve were dogfish and the remaining fifty-five were rays.   Two only, as already mentioned, died from the effect of the anaesthetic, while two others died from suffocation consequent upon my omission to replace the aerating tube in the tank after the specimens had been photographed.

They were killed by being plunged into a mixture of spirit and chloroform and, after a brief stay in this fluid, were evis-cerated.   In this way the blood vessels were practically drained, which greatly facilitated the rapid dissection necessary to expose brain and spinal cord, there being no troublesome effusion of blood from cut vessels within the brain case.   The partially dissected specimens were immersed in a large vessel of fixing fluid (Tellyesnicky's bichromate-acetic mixture) and the further dissection required to expose the greater part of the spinal cord was completed under the fluid.   To dissect away the vertebral column from the hinder part of the spinal cord and the filum terminale is, however, a very delicate operation, which involves considerable risk of damaging the nervous system.   The exposure of the spinal cord was, therefore, carried only to within a couple of inches of the end of the tail.   Behind this point I was con-tent to strip away most of the skin and muscles, about half an inch at the actual extremity being left quite untouched.   In the case of the dogfish the last inch (or even more) of the tail was left intact.

The preparation of the series of sections proved unexpectedly difficult.   In general, a piece of the tail, about an inch in length, was removed—this piece including the point of experimental lesion—and prepared for sectioning.

My intention was to cut this terminal piece sagittally in order that the point of experimental incision and a considerable length of the filum terminale before and behind this point might be seen in one and the same section.   To avoid risk of damage to the sinus terminalis it was found expedient to retain undisturbed the skin upon the last half inch or so of the tail and the terminal piece, therefore, contained the bases of numerous spines embedded in the skin, and separated from the axis of

partly calcified cartilage by particularly tough connective tissue with contained fin-rays. These several structures became greatly indurated during the prolonged paraffin embedding which was found to be necessary. Moreover, the various tissues contracted unequally during this process with the result that despite many precautions a very troublesome crumpling was often produced.

This was most in evidence near the actual extremity of the tail and thus affected, principally, the region behind the incision so that, while it was usually easy to determine if the fiber had retracted backwards from the lesion it was sometimes extremely difficult to certainly recognize the contracted piece of fiber. Especially was this the case when a considerable infiltration of blood into the sinus terminalis had accompanied or followed the recoil of the fiber.

In such sections, the filum terminale appears as a number of isolated pieces, often cut quite obliquely and a diagrammatic sagittal section through the sinus terminalis, such as that seen in text-figure 3, was but rarely obtained.

Apart from this crumpling the tail usually becomes bent at the place where the incision was made, so that the lengths of filum terminale before and behind the incision rarely lay in the same plane, notwithstanding that weights were used during the process of embedding to keep the tissue as nearly flat as might be. In front of the experimental incision the crumpling was less noticeable, the vertebral axis being more rigid, and the muscular and other soft tissues liable to contraction having been, for the greater part, removed. Nevertheless, even here, a certain curvature almost invariably occurred. Further, the greater hardness of the cartilage in this region often caused the sections to cut very unevenly. This irregularity could be largely avoided, it was found, by cutting rather thick sections (not less than 30 μ). The lumen of the central canal, however, in the hinder part of the spinal cord of the rays examined has a diameter which rarely exceeds 30 μ and in such sections, therefore, the whole of the central canal may be included within the thickness of a single section or a relatively thick layer of overlying tissue may seri-

ously obscure the lumen, and a structure so slight as is Reissner's fiber normally can scarcely be distinguished with certainty, if viewed through the thickness of the epithelial wall of the filum terminale. In the swollen or spirally twisted condition the fiber becomes much more conspicuous, it is true, but even so there is still considerable difficulty in making out details. In order, therefore, to make reasonably sure of recognizing the fiber, the sections ought not to have a thickness greater than 20 μ. In such sections the fiber, if present, would be likely to appear as a well defined thread in an open canal and even if the sections should chance to include also an underlying or overlying layer of epithelium, this would almost certainly be quite thin.

Accordingly, the attempt was made to cut the tails in sagittal sections of 20 μ in thickness, the resulting series consisting generally of comparatively thin sections alternating with others considerably thicker, the latter often permitting the presence (and extent) or the absence of the fiber to be ascertained, details being filled in from the thinner sections.

As already observed, the almost invariable distortion of the material led, very generally, to parts of the filum terminale being cut very obliquely (fig. 26). Thus it happened sometimes, even where all the sections of a series were thick, that parts of the lumen of the central canal were exposed clearly to view. On the other hand, even where moderately thin sections had been obtained, there was occasionally some difficulty in deciding whether or no Reissner's fiber was present. In experimental material in which the fiber has been broken, the relaxed fiber may frequently be found lying closely against the surface of the cells which line the central canal. This epithelium has a clear and highly refractive internal border which stains, with borax carmine, a delicate pink, precisely like a lightly stained Reissner's fiber. A very slight alteration of the focus of the microscope produces, along the cut edge, the effect of a double line and gives rise to an appearance which may readily be mistaken for the fiber lying in juxtaposition, optically or actually, with the epithelial surface. It has been found impossible, in some cases, to be absolutely certain whether one is viewing the cut internal edge of this epi-

thelium, or Reissner's fiber lying against it or beyond it. Usually, however, the relaxed fiber does not lie, everywhere, in a perfectly straight line and, if one is actually dealing with the fiber, a careful tracing of the central canal will almost always show the fiber, sooner or later, turning centrally away from the wall of the central canal (fig. 27) and standing for a longer or shorter stretch as a distinct and free central thread.

There would be less difficulty, perhaps, in certainly recognizing the fiber if it invariably maintained its normal thickness (2 μ to 3 μ in the rays) or swelled, as it may do after being cut, to as much as 8 μ or 10 μ in diameter. Not altogether infrequently, however, the fiber appears extremely fine, of a thickness which I estimate to be less than 0.3 μ. Of such a diameter is the fiber in early development in larval cyclostomes and amphibians, and I can only conjecture that the occasional occurrence in these small rays of this delicate fiber is an indication that there has taken place a retraction of the fiber so extensive that repair has taken on the character of a completely new growth which is at first much thinner than in the adult state.

In yet other cases the fiber may be wanting in the region examined but there may be found, lying centrally, a shadowy structure which seems to be a hollow cylinder (fig. 18) whose diameter is considerably greater than that of a much swollen fiber. Were it not that a swollen and displaced length of fiber often lies nearby, I should have been disposed to regard this structure as the product of the disintegration of the fiber. Possibly it represents a film of coagulated cerebro-spinal fluid which has formed around a swollen and gradually withdrawing fiber.

The tissues were stained (in bulk) with borax carmine. Double staining was soon abandoned as it was found that parts of thick sections were at times imperfectly fastened to the slide and were liable to be lost in the staining or decolorizing fluids and it was most important that no parts of Reissner's fiber should be lost in this way. In the few cases in which double staining was resorted to, the second stian was invariably picro-indigo-carmine.

One other point must be mentioned here. In the ray the actual position of the terminal sinus varies slightly, it was found, in different individuals. In the case of the specimen of Raia blanda figured (text-fig. 3) this terminal chamber extended downwards *behind* the extremity of the notochord, which is, I believe, the strictly primitive condition. It occurs, however, less frequently in this position than might be expected, and in many cases it lies altogether dorsal to the notochord, not always extending even to the posterior extremity of that structure. Whether there has been some mutilation in these cases or whether on the contrary there takes place, normally, a certain amount of resorption of the tissue of the terminal filament, I can not decide.

In some teleosts I have found what are, almost certainly, stages in the disappearance of the postero-ventral (post-chordal) part of the neural tube. I find, moreover, that the corrugation of the hinder end of the filum terminale in small rays which I have described ('12, p. 423) as so strongly suggestive of neuromeric constriction, is likewise frequently met with in the vanishing vestiges of the filum terminale in the region of the disappearing tail in the recently metamorphosed anuran.

While these facts suggest that the variation in position of the sinus terminalis of the ray may be due to some extent to the absorption of tissue in this region,[1] the possibility of mutilation must not be ignored. The actual end of the tail of the ray is soft and not protected by spines, and specimens which have suffered quite considerable mutilation are by no means rare. The terminal sinus, too, in those specimens in which it lies wholly dorsal to the notochord (fig. 19) rarely shows that bulbous expansion which is seen in examples in which the sinus terminalis has the postero-ventral position (fig. 20) but has quite a marked resemblance, in shape, to the secondary terminal sinus which I

[1] That an absorption of tissue in this region does occur in rays is suggested by Beard's statements ('96, p. 55, footnote 2), that the young (Raia radiata) immediately prior to escape from the egg case are shorter by a centimeter or so than embryos a month younger. Some of my own specimens which were six inches or less in length must, almost certainly, have been quite newly escaped and the process of resorption was possibly incompleted.

have found produced as the result of my experiments ('12, text-fig).

Be the reason for this variation in position what it may, it has a certain importance in this investigation, for in one or two cases where the sinus terminalis lay unexpectedly far forward, the incision (which was made in the postero-ventral region of the tail, being planned to break the fiber actually in the sinus terminalis) missed the terminal filament altogether.

Of young dogfish, only recently emerged from the egg-case, I have had no material but in the adult there appears to be little variation in the position of the terminal sinus.

In several cases, both dogfish and rays, the cut was made in the region of the terminal filament but just a trifle too far dorsally, and the sections show that, although the cut penetrated the neural canal, the filum terminale and surrounding pia mater escaped damage.

Such specimens in which the experimental incision failed to break the fiber served well as control specimens. Other control specimens were simply anaesthetized without undergoing the usual operation. These latter on recovery behaved in perfectly normal manner.

IV. OBSERVATIONS UPON THE LIVING ANIMAL

1. Upon normal material

The experiment carried out in 1910 had almost immediately directed my attention to the fact that a frequent, if not an invariable, consequence of the operation was the assumption by the subject of the experiment of a very distinct attitude while at rest. Accordingly, during the time spent at Plymouth both in 1910 and 1911, while the experiments were going on in the laboratory, very constant and careful attention was given to the numerous normal specimens which were kept in confinement in the adjoining aquarium. Control specimens, too, were kept under observation in small tanks in the laboratory under conditions precisely similar to those in which the subjects of the experiments were maintained.

It was found that the normal dogfish would, after a period of activity, settle indifferently upon any part of the aquarium floor apparently neither shunning nor choosing the well lighted parts of the tank. Whether, however, they came to rest upon the floor of the tank or upon a rocky ledge in the aquarium, it was observed that they almost invariably settled in some position which gave room for the body to stretch out freely with the tail extended horizontally in the line of the long axis of the body. In such a position (figs. 1, 6) the wedge-shaped head lies with its ventral surface lifted from the floor, but the long axis of the brain has an approximately horizontal position. The trunk, from the branchial region almost to the end of the pelvic fins, lies slightly flattened ventrally against the supporting surface. The pectoral fins are disposed nearly horizontally outwards and backwards. The anal fin is bent over, near its base, sharply to one side so that the actual ventral surface of the animal, behind the pelvic region, is supported just clear of the bottom. Behind the anal fin, however, in which region the trunk tapers off into the tail, the ventral surface no longer touches the bottom but is supported well clear of the tank floor. The caudal fin rests upon the bottom so lightly that its flexible ventral border is scarcely bent. In this attitude, which was found to be invariably assumed by fish confined in the small tanks, the long axis of the central nervous system (which coincides with the position of Reissner's fiber) is maintained, practically, in the horizontal plane. Only at its hinder end, in the heterocercal tail, is this axis slightly upturned.

In the aquarium, in which an attempt is made to reproduce more nearly the natural condition, the bottom is frequently uneven. Whether, however, the fish settles upon the roughly level floor or perches itself upon some jutting rocky shelf, it will be found to maintain the posture described. Upon an uneven supporting surface it will be seen that the body bridges stiffly the gaps between inequalities of the surface and the tail maintains its nearly horizontal position even if there be no contactual surface beneath the caudal region. It is not unusual to see a dogfish resting with the trunk supported upon a rocky

ledge and the tail projecting out stiffly. This is not to be attributed to a natural rigidity of the tail, for this region of the body is peculiarly flexible, and it must be assumed that the posture is maintained by muscular effort.

At times, however, dogfish will wedge themselves into crevices between the rockwork in a nearly vertical position, but even then they maintain a posture in which the long axis is approximately straight.

As will appear, a quite different attitude is assumed by specimens in which the fiber of Reissner has been accidentally or otherwise injured. Indeed, specimens which have received injury resulting in the breaking of the fiber can be easily recognized by the attitude in which they rest.

The normal attitude of the rays is strictly comparable to that just described for dogfish. These animals will, in confinement, settle, apparently indifferently, either upon a horizontal or a smooth vertical surface. While, however, the rays may often be seen adhering to the smooth surface of the wall of the tank, or the glass front of the tank, they appear unable to maintain themselves for long in this position. In the larger tanks and aquaria they seem to exhibit a preference for smooth and level horizontal surfaces.

In either case the whole ventral surface (including that of the head and flattened tail) is applied to the supporting surface (fig. 7). The snout, it is true, may be very slightly lifted from that surface (fig. 10). The flexible tail stretches backwards, its long axis being a continuation of that of the trunk.

2. Upon experimental material

I propose, here, to give a general outline of the reaction observed in the subjects of the experiments in order that the significance of the various experiments, a detailed account of which is given in the succeeding section, may be more readily appreciated.

In the subject of the experiments, recovery from the anaesthetic occurred usually within a very few minutes and was fre-

quently followed by a period of marked activity. In this case the animal would dash about the tank, commonly blundering heavily into the confining walls. This phase rarely endured for long, but gave place to a quiescent stage in which the animal apparently exhibited a preference for the darker part of its tank. Settling down, it might remain inactive for comparatively long periods, moving only when disturbed. In other cases the specimen, recovering from the anaesthetic, passed directly into this lethargic condition. I imagine that this difference in behavior was due to the varying degree in which the animal had been affected by the anaesthetic, a slight degree of insensibility being marked by the erratic activity when volition was recovered.

Be this as it may, the assumption of some posture of the body unlike that which I have described above as normal, was frequently manifested very soon after the quiescent stage was reached. In some cases it appeared within ten minutes of the operation. Both the head and tail would be gradually lifted until the long axis of the body, from being a straight line would become markedly curved (figs. 2–5). The tail was, in general, sharply upturned from its base, while the trunk region was uplifted upon the pectoral fins from a region just behind the head. In the rays, owing to the great development of the pectorals, this appears to give rise to a transverse curvature of the anterior part of the body, as seen from in front (figs. 9, 13, 14).

There may be also a distortion of the long axis in the horizontal plane, the trunk and tail being bent several times from side to side (in some of the dogfishes) or with a single sharp bend of the hinder part to one side (rays and dogfish).

It is probable that a disturbance of the poise of the body exists, likewise, while the animal is in motion. It is, however, very difficult to be sure of this. In some of the dogfish, certainly, uniform undulation of the body in swimming seemed to be replaced by a less even movement which is perhaps best described as a wriggling action.

These reactions did not always make an appearance quickly after the operation. In some cases their advent was delayed for days even, and in yet others, as will be seen from the detailed

record given below, they never appeared at all. The explanation of these apparent exceptions must be deferred until after the account of the microscopical examination of the experimental material.

The duration of the reaction also varied considerably, persisting in some cases for a few hours only, while in others it endured for several days. In a few cases it appeared to be intermittent.

V. A SUMMARY OF THE RECORD OF THE EXPERIMENTS AND AN ACCOUNT OF THE EFFECTS UPON REISSNER'S FIBER

A. Scyllium canicula

2. The experimental incision was made at noon on July 7, 1910. The specimen quickly recovered and, although somewhat sluggish, appeared to swim normally. At rest, its body was bent slightly but otherwise the posture seemed normal. No change was observed until July 11, when the ventral border of the caudal fin was seen to be lifted slightly (about half an inch) from the tank floor. The animal became more sluggish and, if disturbed, soon returned to rest, exhibiting an apparent preference for the darkest corner of its tank, which rendered observation more difficult. The tail rested, moreover, against a sloping part of the tank where wall and floor met. It was impossible, therefore, to be sure whether the tail was really slightly lifted by muscular effort or merely upraised on account of the elevation of its support. The whole body, however, was seen to be considerably curved. On July 13 and 14 the fish was more restless and upon the 15th, when seen at rest, the long axis of the body was disposed in a straight line (for the first time since July 8). During the following day it was noticed that the body of the fish was once more bent from side to side in long wavy curves, but with the tail, as before, supported upon the sloping part of the tank. Next day, however, it was found resting well away from the back of the tank and the tail was uplifted, a clear two inches, from the floor. By midday on the 18th this reaction was still more marked and the hinder part of the trunk and tail were bent sharply to one side. Throughout the two succeeding days this reaction was pronounced. On July 21 the fish had reverted to an earlier posture, with the tail supported against the sloping part of the floor, but by midday it was once again well out in the tank with the tail held well off the floor. On the next day the reaction was less marked though the body was still bent. During July 23 the reaction was scarcely discernible and later in the day, when it was decided to kill the specimen, the fish appeared normal. It showed very marked activity in its attempts to avoid the net, swimming with a wriggling movement (the head and forepart of the body being twisted quickly from side to side). This action in swimming had been

noticed on several previous occasions. This specimen, then, gave a marked reaction lasting for 13 days. Duration of the experiment 16 days +.

The sections showed that, in front of the incision, a secondary sinus terminalis had been produced into which Reissner's fiber is seen to extend and, in contact with the hinder (meningeal) wall of which, it flares. Apparently it has just become attached thereto (fig. 31). Behind the incision the fiber has apparently entirely disappeared.

9. This specimen, for nearly a week after the operation (performed on July 12), showed a curious restlessness, not once being observed at rest until the morning (10 a.m.) of July 18. This activity was followed by an equally marked lethargy. The specimen took up a position in the darkest corner of the tank, where it lay with the body bent upon itself at a sharp angle and the tail supported against the sloping part of the tank. Not until July 22 was the specimen seen, in repose, away from the wall of the tank when it was found resting with the end of the tail slightly lifted: the flexure of the body was nearly straightened out. It was killed on July 23 the experiment having lasted 11 days.

In the sections the fiber (in front of the lesion) is found to extend backwards nearly to the place where the filum terminale was severed. It is probable, therefore, that the fiber had nearly recovered from the effect of the operation but the experiment was ruined by an accidental cut made, far forward in the trunk region, when exposing the spinal cord. The fiber in the piece examined is much swollen and continuously twisted (text-fig. 2), undoubtedly due to a (backward) retraction from this distant cut.

20. The incision was made at 11.15 a.m. on August 3, 1911, and, by noon, the tail was lifted slightly so that the lower border of the caudal fin no longer rested upon the tank floor. When disturbed, the fish swam with a quick wriggling action (cf. 2) and came to rest in a curious attitude in a corner of the tank, the anterior part of the trunk being poised vertically, supported by the adjacent walls of the tank, while the posterior part lay out horizontally upon the tank floor (cf. the ray, fig. 11). After being again disturbed, it once more came to rest in this peculiar attitude and so remained until 3 p.m. at which time it was again compelled to move. It was observed to swim in quick rushes, even leaping partly out of the water, and the wriggling movement was very noticeable. Ten minutes later it had settled down with the end of the tail slightly lifted but resting lightly against the tank wall. It was disturbed yet again and was subsequently induced to settle well away from the walls of the tank and the tail was then seen to be held at least an inch and a half from the floor, and it continued in this attitude until 4.15 p.m. when it was accidentally disturbed. During the next half hour it was repeatedly set in motion by the movements of another dogfish which shared the tank. It settled down six several times in the same attitude (with head and tail lifted) once or twice essaying the half vertical position which it had

assumed earlier.   By 4.45 p.m. the tail was lifted more than two inches from the floor.   The specimen was then driven about the tank and compelled to swim actively for several minutes and then removed and killed.   There was in this case a well marked reaction which endured for the entire period of the experiment—$5\frac{1}{2}$ hours.

In front of the lesion the fiber has completely withdrawn from the piece of terminal filament and spinal cord examined.

21. The incision was made at 11.40 a.m. August 3.   Upon recovery from the anaesthetic the specimen adopted the normal attitude.   It was killed at 10.30 a.m. August 6, having given no apparent reaction during the three days of the experiment.

The sections are poor but show that the incision missed the terminal filament and thus failed to break the continuity of the fiber which is seen to be of normal diameter and to lie tautly stretched.

22. Within 10 minutes of the operation (performed at 10 a.m., August 4) a marked reaction appeared, the lower border of the caudal fin being lifted a clear two inches from the tank floor.   The animal was sluggish and, after being disturbed, reverted always to this attitude.   It was twice photographed later in the afternoon but the reaction had then become less marked (figs. 2, 3) but continued as shown until the specimen was killed at 5 p.m.   Duration of the experiment 7 hours.

The fiber has apparently been withdrawn forward from the lesion completely beyond the anterior limit of the piece of spinal cord sectioned.

23. The incision was made at 10.10 a.m. August 4, but was followed by no apparent reaction and the specimen continued normal until it was killed on August 22.   It was photographed on August 7 (fig. 6). Duration of the experiment 18 days 8 hours.

The sections show that the cut failed to penetrate the neural canal and the normal Reissner's fiber may be seen lying tautly stretched in the central canal of the undamaged terminal filament.

24. The incision was made at 4 p.m., August 4, and the usual reaction was noticed within half an hour of the operation, the caudal fin being lifted two inches or more.   It was, however, less sluggish than the subject of the preceding experiment and frustrated all attempts to obtain a photograph during the early days of the experiment.   The reaction continued uninterruptedly until the evening of August 8, the photograph (fig. 5) being obtained about midday on August 6. From the 9th onwards the reaction appeared intermittently and during the whole of the 10th the caudal fin was observed to be resting lightly upon the floor of the tank though the head was still somewhat raised. Late in the evening of the 15th and again at noon on the 18th the tail appeared slightly lifted for a while, but for the most part the reaction rarely appeared for any length of time after the morning of the 14th August (the eleventh day of the experiment).   The specimen was notably sluggish during the later stage of the experiment and passed most of the time in a corner of the tank, with the tail supported upon

the sloping surface there. It was killed at 6.30 p.m., August 22. Duration of experiment 18 days $2\frac{1}{2}$ hours.

The severed (hinder) portion of the terminal filament had not markedly disintegrated but the short length of Reissner's fiber separated by the incision has altogether disappeared. There is visible some disorganization of the terminal filament in front of the lesion, but a little in front of the point where the cut was made the lumen of the central canal seems to have been widened somewhat, perhaps, to form a secondary sinus terminalis. Stretching backwards to this point, there is seen a flimsy wrinkled and fibrillate structure which is, I believe, the expanded hinder end of Reissner's fiber.

34. The incision, which was made at noon, August 14, was followed within a quarter of an hour, by a distinct reaction (fig. 4). This continued and was well marked at 3 p.m. when the specimen was killed. Duration of experiment 3 hours.

In front of the region where the experimental incision was made, the fiber is found retracted and swollen with some spiral twisting. Behind the point of injury the fiber is markedly swollen and appears fibrillar.

33. The incision was made at 12.05 p.m., August 14, and by 1 p.m. a slight reaction had appeared, but for the greater part of the day, the animal rested with the tail turned over upon its side. The whole body was strongly curved. During the following day this same attitude was largely maintained but at times the tail was seen to be lifted considerably. The specimen was killed at 8.45 p.m., August 15, the duration of the experiment thus being 1 day $8\frac{3}{4}$ hours.

Reissner's fiber is found swollen, retracted forward from the region of the experimental incision and, at the free end, is slightly spirally wound. Traced forwardly this spiral becomes a very open one and the fiber passes into a comparatively straight course. It probably represents a stage in unwinding.

43. The incision was made at 11.20 a.m., August 18, and was quickly followed by the usual reaction, the head being well raised. By noon the tail, also, was well lifted and the head still further raised. At 7.15 p.m. when the specimen was killed, the reaction appeared less pronounced. Duration of experiment 8 hours.

In front of the lesion, Reissner's fiber had disappeared entirely from the length of tail examined. Behind the incision also, the fiber has evidently contracted, the canal being devoid of fiber nor can the contracted piece be certainly recognized.

44. The incision was made at 11.45 a.m., August 18, but no reaction appeared either upon this or the following day. The specimen was killed at 8 p.m., August 19. Duration of experiment 1 day $8\frac{1}{4}$ hours.

The fiber though severed has apparently been gripped by the adpression of the walls of the terminal filament and there has been no retraction of the fiber in either direction (fig. 30).

B. Raia blanda

3. This specimen was one which failed to recover from the anaesthetic. Some 2 to 3 hours after the operation it appeared to be dead, the central nervous system, therefore, was partially exposed and preserved.

The sections show that the incision severed the fiber but at the same time apparently pinched together the walls of the terminal filament sufficiently to hold the cut ends. Behind the incision, therefore, the fiber is found, stretching backward from the region of the lesion to the sinus terminalis. It is somewhat swollen, the swelling becoming more marked as the terminal sinus is neared and, actually within the terminal chamber, it becomes greatly swollen and coiled. The terminal plug is obscured by this retraction and cannot be certainly identified (text-fig. 4).

In front of the lesion Reissner's fiber is found, everywhere in the length of the terminal filament examined, much swollen and most remarkably coiled, all the later stages of spiral winding (short of the production of actual tangles) being found in this short extent of central canal (text-fig. 2). Regions in which the fiber is simply twisted alternate with others in which the coiling is quite complicated and it is probable that the original (uncontracted) length of the fiber included in the piece examined was many times that of the length (about $\frac{3}{4}$ inch) of the containing central canal. The evidence suggests, therefore, that there must have been in progress, at the time the incision was made, a very definite retraction of the fiber in a backward direction from a point well in advance of the experimental cut. This cut clearly checked further retraction behind the lesion, but in front the retraction probably continued until it was stopped by the hardening action of the fixing fluid several hours subsequent to the operation.

4. The incision was made at 10 a.m., July 8, and was followed by a quick recovery. Thereafter, the fish swam about with its tail turned dorsally. Six hours later, when seen at rest, it was noted that its tail was turned sharply to one side and that the extremity was raised at least an inch. The tail was still lifted at 9.30 a.m. next day but later this peculiarity was less pronounced. The specimen was killed at 3 p.m. Duration of experiment 1 day 5 hours.

The sections are poor and very obliquely cut. A considerable clot occupies the central canal for some distance in either direction from the experimental lesion. Behind the region of the incision I have failed to recognize Reissner's fiber but in front it can be made out vaguely, apparently lying somewhat slackly against the epithelial lining of the central canal and with some trace of irregular swelling and coiling (fig. 16).

7. The incision was made at 10.30 a.m., July 9, and was quickly followed by an elevation of the end of the tail, the whole tail being turned slightly to one side (the left). By July 11 the fish appeared to have become normal, excepting that it continued to exhibit a prefer-
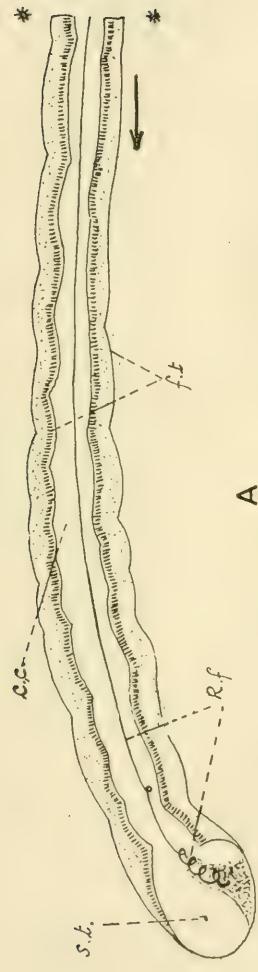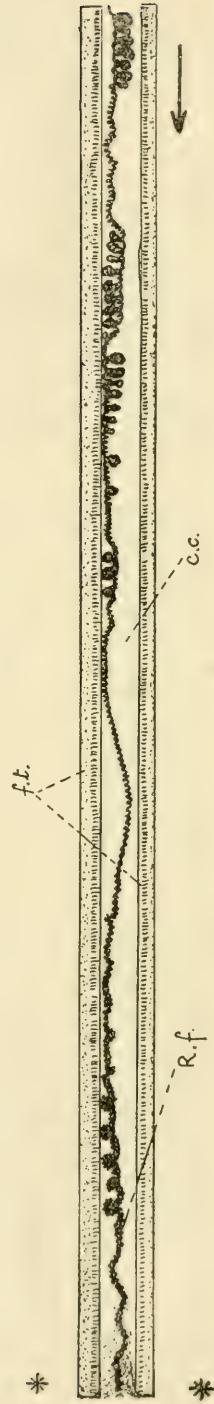
Text-fig. 4 A slightly diagrammatic median sagittal section through the filum terminale of Raia blanda (3) behind, 4A, and in front, 4B, of the experimental lesion. The arrows indicate the direction in which recoil of the fiber had taken place. *c.c.*, central canal of the spinal cord (and terminal filament); *f.t.*, filum terminale; *R.f.*, Reissner's fiber; *s.t.*, sinus terminalis; **, indicate the region of the incision.

ence for the dark corner of its tank and adopted the somewhat unusual action in swimming noted in no. 5 (Raia clavata). During the 11 succeeding days, it was observed at frequent intervals but was apparently normal throughout this period. On July 22 it was killed Duration of experiment 13 days +.

This tail was examined by means of sections cut transversely which proved to be quite unsuitable for the purpose of this investigation. The fiber is found in the more anterior sections, where it appears not markedly swollen and has apparently nearly made good any retraction which may have taken place after the operation.

8. The incision was made at 10 a.m., July 13. By 10.30 a.m. the specimen had completely recovered from the anaesthetic and had attached itself to the (vertical) glass plate forming the front of the tank, the tail being lifted slightly and turned to the left. This condition persisted for an hour or so but by midday the ray appeared normal. It was killed at 11 a.m., July 14. Duration of the experiment 25 hours.

There was no considerable retraction of the severed ends of the fiber, in either direction from the lesion, these being entangled apparently, in the clot which occupies the central canal for some distance. From this clot the fiber may be traced tautly stretched and of normal diameter.

10. The incision was made at 11.30 a.m., July 13, and was followed very quickly by a marked uplifting of the tail. An hour after the operation the ray was found adhering to the wall of the tank with the tail swung out dorsally and to the left. The reaction continued to be marked during the three following days. By the morning of July 17, however, the ray was seen with the tail carried normally and, thereafter, the specimen appeared normal until July 23 when it was killed. Duration of experiment 10 days.

For some distance in front of the experimental lesion, the central canal is found empty of fiber. The free end of the fiber is found, about half an inch in front of the lesion, swollen and thrown into a loose tangle (fig. 26), from the anterior end of which the fiber emerges much less swollen and fairly straight. No part of the fiber shows any trace of spiral twisting.

11. The incision was made at 10 a.m., July 20, with a fine knife from the right side, care being taken not to penetrate completely through the tail. The usual reaction did not appear, but there was some displacement of the right pectoral fin which was brought up sharply dorsally. Next morning the ray appeared entirely normal. It was killed at 4 p.m., July 22. Duration of experiment 2 days 6 hours.

The sections show that the incision missed the filum terminale and the fiber, therefore, remained unbroken.

19. The incision was made at 4.10 p.m., August 2. The specimen was kept under observation until it was killed on August 9 but during the whole of this time nothing unusual in its behavior was noted. Duration of experiment 6 days 20 hours.

The filum terminale behind the lesion appears empty of fiber but an indistinct mass, which is apparently a tangled heap of fiber is seen in the sinus terminalis. In front of the lesion the fiber is slightly withdrawn, the end being swollen and somewhat spirally coiled.

29. The incision was made at 4.20 p.m., August 7, and the ray was killed at 7.15 p.m. on August 10, no reaction having appeared in the meanwhile. Duration of experiment 3 days 3 hours.

The sections establish that the incision failed to sever the filum terminale and the fiber which is unbroken maintains its normal diameter and is seen tautly stretched.

41. The incision, made at 5.55 p.m., August 17, was followed, very quickly, by a reaction. The snout was lifted markedly and the whole body was arched up. The ray was disturbed several times but invariably returned to rest in the same attitude. By 8.30 p.m. the reaction had become less pronounced and by noon next day, when the specimen was killed, it was much less marked. Duration of experiment 18 hours.

In the terminal piece of the tail, Reissner's fiber is found slack, swollen and retracted for some distance from the region of the experimental lesion. Another piece of the spinal cord, taken some considerable distance in advance, showed the fiber very slightly slack and little swollen.

49. The incision was made at 11.15 a.m., August 21. A marked reaction very quickly appeared, affecting the pose, both in swimming and at rest. At noon, the snout and tail were down but the body remained curiously humped up. The specimen maintained this attitude until it was killed at 12.45 p.m. Duration of experiment $1\frac{1}{2}$ hours.

A conspicuous clot has formed in the region of the lesion and extends into the central canal both before and behind this point. Reissner's fiber is seen extending backwards from this spot as a swollen, loose and slightly knotted thread. In front of the incision, the fiber emerges from the clot (fig. 22) markedly swollen and coiled interruptedly, in which condition it continues throughout the entire length of the piece of spinal cord examined. The penultimate piece reveals the fiber still more swollen and more markedly twisted. It is clear, therefore, that although there has been no withdrawal, in either direction from the region of the experimental incision, the fiber was, nevertheless, undergoing a marked contraction. The only possible explanation was that the fiber had been broken farther forward and that a backward recoil had been set up, that having begun probably at or about the time of the operation. To test this point, another piece of spinal cord was taken from a place well forward in the trunk. The sections showed that, here, the central canal was perfectly devoid of fiber, a flimsy hollow cylinder of coagulum (?) occupying the center of the canal (fig. 18).

51. The incision which was made at 11.30 a.m., August 21, was not apparently productive of any reaction. The specimen was killed at 5 p.m. on the same day. Duration of experiment $5\frac{1}{2}$ hours.

The tail had clearly been truncated earlier in life but had completely healed and a secondary sinus terminalis had been formed. The experimental cut failed to break the fiber which is seen of normal size and tautly stretched.

52. The incision was made at noon and was followed by a scarcely perceptible reaction. The ray was killed at 3.30 p.m. Duration of experiment $3\frac{1}{2}$ hours.

The fiber was severed by the incision but the free ends, which are slightly knobbed and swollen are entangled in a clot and thus, presumably, retraction has been prevented.

53. The incision was made at 12.05 p.m. and was quickly followed by a fairly definite reaction which, however, was not evident at 2.30 p.m. when the specimen was killed. Duration of experiment $2\frac{1}{2}$ hours.

The fiber is seen cut and slightly slackened but the free ends have been withdrawn only for a short distance from the lesion.

62. The incision was made at 8.30 p.m., August 21, and was seen to be followed by a marked swimming reaction but the specimen was not seen in repose, after the operation. It was killed at 10.30 p.m. Duration of experiment 2 hours.

The sections are poor but serve to show that the filum terminale was cut. No fiber can be made out in such parts of the central canal as I have been able to examine. It is probable that the fiber has retracted forward, beyond the anterior limit of the piece of tissue sectioned.

63. The incision was made at 11.10 a.m., August 22, and was followed by a marked swimming reaction. At 11.40 a.m. it settled down but the tail was not displaced. It was killed immediately. Duration of experiment 30 minutes.

The fiber had been cut and had, apparently, retracted forwardly, completely from the filum terminale in the piece of tail examined.

66. The incision was made at 11.35 a.m. and was followed by a marked reaction. The ray was killed at 12.20 p.m. Duration of experiment 45 minutes.

The fiber was cut and had retracted some distance forward. In the sections it may be seen lying slackly in an undulating course but is not appreciably swollen.

C. Raia clavata

5. The incision was made at 10 a.m., July 8. By 5 p.m. the hinder part of the tail was seen to be lifted and this reaction was manifested throughout the evening and became still more marked next day. On July 11, the specimen appeared very lethargic and, on every occasion after being disturbed, returned to rest in the darkest part of its tank. In swimming, the specimen would remain poised nearly vertically, with a curious hovering movement, for 10 minutes or more at a time, its tail being turned sharply dorsally. At rest, so far as could be seen, the tail was disposed normally, but next day it was held uplifted for

several hours. On July 13, the tail was seen turned to one side and supported upon the side of the tank, the animal being very sluggish. Next morning, appearing normal, it was killed. In this case, then, the reaction appeared about 6 hours after the operation, was exhibited continuously for 24 hours and intermittently during the three following days. Duration of experiment 6 days.

In this case the tail piece was cut transversely, which sections it was found are quite unsuitable. Behind the incision, the fiber seems to have largely withdrawn into the sinus terminalis in which a coiled mass can be recognized. In front of the lesion, the fiber, somewhat swollen and lying unevenly (fig. 24), appears to extend backwards almost to the point where it had been broken.

6. The incision made at 9 a.m., July 9, was followed very quickly by an uplifting of the tail which amounted to as much as $2\frac{1}{2}$ inches or even 3 inches from the tank floor (the specimen being only 9 inches in length). The head was also raised, the tip of the snout being lifted at least 1 inch, so that the long axis was very markedly curved. In addition, there was a transverse flexure of the body, the lateral borders of both pectoral fins being sharply upturned, also. By 2.30 p.m., the trunk had become flattened and the ray had settled down normally excepting that the tail was still raised and at a sharp angle to one side. Next day the tail had resumed its normal (horizontal) position. In its marked lethargy and in adopting an unusual attitude in swimming (when disturbed) it resembled the preceding specimen (5). On July 12, at 10 a.m., the tail was again seen to be well raised: disturbed, the specimen would rise to the surface and float in a nearly vertical position for as many as 20 minutes at a time. On each occasion, it returned to a vertical position of rest. On July 13, it was observed at rest upon the floor of the tank with its tail displaced to one side (the right) but horizontal. Next day it appeared normal and, during the morning, was killed. In this instance, there was a well marked reaction manifested almost immediately after the operation and continuing for 24 hours or so but thereafter only appearing intermittently. Duration of experiment 5 days +.

The sections are quite unsatisfactory. Behind the lesion, the fiber appears to have been caught by the pinching together of the walls of the filum terminale and has not retracted. In front of the region of the incision, however, the fiber has been withdrawn forward out of the terminal piece sectioned. Sections were prepared of the penultimate piece and these revealed a very delicate filament lying slackly near the anterior end of this piece.

15. The incision was made on August 2, at noon, the needle being thrust into the very extremity of the tail. There was no reaction and the ray was killed at 7.30 p.m. Duration of experiment $7\frac{1}{2}$ hours.

In this specimen the sinus terminalis does not extend downward behind the notochord but lies wholly dorsal to that structure. The cut, therefore, failed to penetrate the sinus terminalis and the fiber was undamaged.

16. The incision was made at 12.15 p.m. When the ray was returned to its tank, the tail was seen to float slightly off the floor but with the visible return to consciousness, the ray took up the normal position. The specimen appeared very inert and, upon examination made next day, it was found that the vertebral column had been completely broken at a point some distance from the end of the tail. The specimen was killed at 10.30 a.m., August 23, some 22 hours after the operation.

Sections through the end of the tail showed that the hinder end of the spinal cord had already largely degenerated, obviously as the result of the accident which had broken the tail.

17. The incision which was made at 2.30 p.m., August 2, was followed very quickly by a curving up, lengthwise, of the body and snout. This reaction persisted throughout the remainder of the day. Next morning the specimen was found in the normal attitude. It was killed at noon. Duration of experiment $21\frac{1}{2}$ hours.

The sections show that the fiber had withdrawn wholly from the terminal piece. The penultimate piece of the tail was subsequently sectioned but the fiber was absent from the length of spinal cord included in these sections, also.

18. The incision was made at 2.40 p.m., August 2, but no reaction was apparent. The specimen was kept under observation until noon, August 9, when it was killed. Duration of experiment nearly 7 days.

The sections showed that the fiber was broken by the operation and has, in the small severed portion of the terminal filament, entirely disappeared, while this piece of the terminal filament itself appears to have largely degenerated. In front of the lesion, the fiber stretches backwards practically to the point where it had been cut. Near its free end it is, however, slightly swollen and a little slack and its actual extremity is distinctly fibrillated (fig. 28), the flaring of the extremity suggesting that a terminal plug was in process of formation. There was, when the specimen was killed, no new terminal sinus formed. A little in front of the actual end the fiber is little swollen and runs nearly truly in the center of the canal, surrounded by an extensive blood clot which doubtless prevented the retraction of the fiber.

26. The incision was made at 4 p.m., August 7, but no reaction appeared during this or the three following days. The specimen was killed on August 10, at 6.30 p.m. Duration of experiment 3 days $2\frac{1}{2}$ hours.

Reissner's fiber is seen in the sections as an extremely fine thread stretching forward tautly from the point of experimental incision and there has, apparently, been no retraction.

28. The incision was made at 4.20 p.m., August 7, and produced no apparent reaction. The ray was killed on August 10, at 6.15 p.m. Duration of the experiment 3 days 2 hours.

The fiber has evidently not retracted, in either direction from the point of incision being held, apparently, by the adpressed walls of the terminal filament.

32. The incision was made at 7 p.m. on August 10. Upon recovery, the specimen showed an unusual swimming reaction, then settled down with the tail lifted dorsally. It was killed at 7.40 p.m. Duration of experiment 40 minutes.

The sections are practically worthless, merely establishing the fact that the incision had severed the terminal filament.

36. The incision was made at 6.15 p.m., August 15, and was followed by a very slight uplifting of the snout. The left pectoral fin was also raised. Presently the fish settled normally but later the right pectoral was lifted. The specimen attempted to settle upon the tank walls but failed to maintain this position. At 7 p.m. the ray appeared in no way abnormal and at 7.45 p.m., it was killed. Duration of experiment $2\frac{1}{2}$ hours.

The fiber was cut, but both free ends seem to be entangled in a large clot and there is no evidence that any retraction took place.

37. The incision was made at 7 p.m. and was followed, almost at once, by an elevation of the snout. Like the ray just described, it appeared to prefer a vertical position but was unable to maintain itself upon the tank walls for any length of time, invariably sliding downwards until it was supported by the outwardly (dorsally) bent tail (fig. 11). Any reaction affecting the tail, therefore, was masked, if it occurred. The ray was killed at 6 p.m. on August 16. Duration of experiment 1 day 23 hours.

In front of the place of the lesion a somewhat limited retraction occurred, but the fiber appears to have become caught in an elongated clot which extends for some distance forward along the lumen of the central canal. From the anterior end of this clot the fiber emerges as a swollen and indistinctly spirally wound thread (fig. 23).

38. The incision was made at 11 a.m., August 16, but was not followed by any visible reaction. The specimen was killed at 11.45 a.m. Duration of experiment 45 minutes.

The tail of this specimen had, at some time, suffered mutilation but the wound had completely healed and a secondary sinus terminalis had been produced. The experimental incision had severed the fiber but the cut ends had not retracted, being held, apparently, by the pinching together of the walls of the filum terminale.

39. The incision was made at 11.15 a.m., August 16 but produced no evident reaction. The ray was killed at 5.30 p.m., next day. Duration of experiment 1 day $6\frac{1}{4}$ hours.

There was no forward recoil of the fiber from the point where it was cut experimentally. During the dissection made to expose the spinal cord, however, an accidental cut was made far forward in the spinal cord which evidently broke the fiber in that region and it appears slack and swollen even as far back as this terminal piece.

40. The cut, made at 12.15 p.m., August 16, accidentally removed the end of the tail (a piece about one-sixteenth of an inch in length). The fish took up a vertical position, with the body supported by the out-turned tail (cf. no. 30) which, as already pointed out, masks the

tail reaction, if that were produced. The ray was killed at 1 p.m. next day. Duration of experiment 1 day $\frac{3}{4}$ hours.

The fiber had apparently retracted forward, completely beyond the anterior limit of the terminal piece sectioned.

42. In this experiment, also, the cut (made at 6.05 p.m., August 17) accidentally severed the tail, a piece scarcely one-sixteenth of an inch in length being removed. The specimen assumed the vertical position, both in swimming and at rest. Induced to settle upon the floor of the tank, it remained in a nearly normal attitude, the snout only being somewhat raised. It was killed at 4.30 p.m. on August 19. Duration of experiment 1 day $22\frac{1}{2}$ hours.

The sections are poor and very oblique near the hinder end. Reissner's fiber cannot certainly be made out near the point where it was cut. Further forward it is seen here and there and then appears of normal diameter, lies centrally and is apparently tautly stretched. Probably no retraction took place, but the cut end was gripped by the walls of the filum terminale.

45. The incision was made at noon, August 18. The ray rested in a vertical position but the tail was not deflected from the line of the long axis of the body. It maintained this attitude and was killed next day, no reaction being noted. Duration of experiment 1 day $3\frac{1}{2}$ hours.

In this specimen, the fiber seems to have been prevented from retracting forward by the grip of the adpressed walls of the filum terminale, while behind the point of injury a clot has formed in the central canal and apparently the free end of the severed portion was held by this clot.

46. The incision was made at 12.10 p.m., August 18, and at 2.30 p.m. the end of the tail was seen to be slightly raised. This reaction wore off during the afternoon and the ray seemed perfectly normal next day. It was killed at 3 p.m. Duration of the experiment 1 day $2\frac{3}{4}$ hours.

In this ray, Reissner's fiber is remarkably delicate. It lies a little slackly, apparently, but otherwise shows no sign of retraction. For some distance it lies embedded in an elongated blood clot.

47. The incision was made at 3.30 p.m., August 19. On recovery from the anaesthetic, the fish settled in a corner of the tank with its tail raised several inches and resting against the wall of the tank. Later it was gently moved away from the vicinity of the wall and it then brought down the tail to the tank floor. It was killed at 4.45 p.m. Duration of experiment $1\frac{1}{4}$ hours.

The short, severed portion of Reissner's fiber has not retracted backwardly. In front of the injury, however, the fiber has withdrawn forward completely out of the length of filum terminale examined. Sections of a piece of spinal cord, taken from a region well forward in the trunk, reveal the fiber practically normal in size but lying slightly slackly and undulating.

48. The incision was made at 4.45 p.m., August 19, and was followed by a very slight uplifting of the end of the tail, the lateral bor-

der of the pectoral fins being also slightly upturned. By 7 p.m., the reaction had apparently passed; at 7.30 p.m. the ray was killed.   Duration of the experiment $2\frac{3}{4}$ hours.

The sections are very thick and, excepting that they show that the filum terminale (and, therefore, Reissner's fiber) was severed, are practically useless, affording no information as to the effect of the cut upon the fiber.

54. The incision was made at 12.10 p.m., August 21.   There followed a marked reaction which was still pronounced at 12.55 when the ray was killed.   Duration of experiment 45 minutes.

The fiber had retracted wholly beyond the anterior limit of that piece of the tail which was sectioned.

55. The incision was made at 2.40 p.m., August 21.   The whole body of the specimen became slightly lifted, being supported upon the bases of the pectoral fins.   The tail was held out stiffly, unsupported, in a nearly normal position, its end, however, drooping slightly.   This attitude was maintained until 4.30 p.m. when the specimen was killed. Duration of experiment 1 hour 50 minutes.

The fiber, in this example, is extremely slight in the tail region.   It was evidently broken by the experimental incision but there seems to have resulted very slight displacement of the free end; traced forward from the region of the incision the fiber is seen to lie somewhat slackly against the wall of the central canal.

56. The incision, made at 2.45 p.m., August 21, was quickly followed by a very marked reaction.   The ray lifted itself well up from the floor until it was supported only by the lateral border of the pectoral fins (fig. 14).   The tail was turned up sharply dorsally.   The specimen was photographed in this attitude at 3.15 p.m. and was killed at 3.30 p.m. Duration of experiment 45 minutes.

Behind the injury the fiber is seen swollen and, in the terminal sinus, it is spirally wound but complete retraction was apparently prevented by the formation of a clot which has entangled the severed end.   In front of the lesion four pieces of vertebral column (including more than 3 inches of the spinal cord, or half of its entire length) were sectionized but the fiber had withdrawn forward beyond the most anterior point examined.

57. The incision was made at 4 p.m. but the reaction was not noticed until 7.15 p.m. when both snout and tail were well lifted.   The ray was killed at 7.25 p.m.   Duration of experiment $3\frac{1}{2}$ hours.

In front of the point of injury, the fiber was absent in the length of spinal cord sectioned.

58. The incision, which was also made at 4 p.m., was quickly followed by a well marked reaction, the whole body being lifted upon the pectoral as well as both snout and tail upturned.   The ray was killed at 4.50 p.m.   Duration of experiment 50 minutes.

The fiber had retracted completely beyond the forward limit of the piece of the filum terminale examined.

59. The incision, made at 5.20 p.m., was followed by a reaction as pronounced as that seen in the subject of experiment 56 (fig. 14). The ray was still in this attitude at 7.15, when it was killed. Duration of experiment, a little over 2 hours.

In this example, also, there had been considerable retraction, the fiber not being found in the stretch of spinal cord examined.

60. The incision was made at 5.15 p.m. Within a quarter of an hour there appeared a marked reaction which continued until 10.30 p.m. when the specimen was killed. Duration of experiment $3\frac{1}{4}$ hours.

Here again, the sections showed the central canal, in the length of spinal cord sectioned, completely empty of Reissner's fiber which must, therefore, have retracted forward out of this region.

64. The incision was made at 11.05 a.m., August 22. There followed a marked swimming reaction and, when the specimen settled down, the tail was well raised. The ray was killed at 11.15 a.m. and the partly dissected specimen was in fixing fluid within 14 minutes of the beginning of the experiment. Duration of experiment 10 minutes.

Behind the region of the incision, Reissner's fiber is found lying slackly in the central canal. In front of the injury, there has been some retraction, the fiber lying in loose wavy curves (fig. 27).

67. An incision was made at 10.45 a.m., August 24, and was very quickly followed by a marked reaction which persisted until the specimen was killed at 12.20 p.m. Duration of experiment 1 hour 35 minutes.

The fiber had withdrawn completely beyond the anterior limit of the piece of filum terminale examined.

68. The incision was made at 10.45 a.m., August 24. A marked reaction quickly appeared, the whole body being arched up and supported only upon the lateral borders of the pectoral fins while the tail was sharply uplifted. Two photographs (figs. 13, 9) were taken at about 11.30 a.m. and noon, respectively. Duration of experiment $1\frac{1}{2}$ hours.

In this specimen, also, the fiber has been withdrawn completely beyond the anterior end of the piece of spinal cord sectioned.

70. The incision was made at 12.30 p.m. A distinct elevation of the snout appeared when the specimen had recovered from the anaesthetic but the tail was unaffected. Photographed at 12.45 p.m. (fig. 12), the specimen was killed at 1.10 p.m. Duration of experiment 40 minutes.

The sections are poor, being thick and oblique. They establish however, that the incision just failed to break the filum terminale and, in the one or two sections, in which Reissner's fiber can be made out, near the place of the incision, it appears to lie centrally in a fairly even course. But in a moderately thin section which shows the sinus terminalis quite clearly there are signs of recent disorganization and Reissner's fiber is not present. Much of the lumen of this terminal chamber is occupied by a clot which is certainly not due to an effusion of blood resulting from the experimental cut.

D. Raia microcellata

61. The incision was made at 7.15 p.m., August 21.   At 10.30 p.m
the tail was distinctly raised and remained so until the specimen was
killed at 11 p.m.   Duration of experiment $3\frac{3}{4}$ hours.

Sections prepared through the tail were useless.   The brain was
sectioned, sagittally, and showed the fiber lying in normal position, of
usual size and apparently tautly stretched, so that if retraction of the
fiber took place in the tail region, it had not extended forward to the
head.

In all, serial sections were prepared of sixty-two specimens.[2]
Of these, the microscopical examination showed that in one case
(16) the hinder part of the spinal cord was in an advanced stage
of degeneration due to an accident which must have occurred
at some time prior to the experiment.   The sections through
the region including the point of injury were, in five cases (30,
50, 61, 63, 69), absolutely worthless and two others (32, 48)
were somewhat fragmentary and of value only in establishing
that the experimental incision had severed the filum terminale
(and therefore Reissner's fiber), while in another instance (70)
the sections are, for the most part, very thick and Reissner's
fiber can be but doubtfully distinguished.   In this case the
experimental incision did not penetrate the filum terminale.

Sufficiently satisfactory sections were obtained, therefore, in
fifty-three examples.   Of these Reissner's fiber shows a most
remarkable coiling in two cases (3, 49) which must be attributed
to the breaking of the fiber very shortly before the experiment.
In the former of these, moreover, the specimen never recovered
from the anaesthetic and afforded, therefore, no reaction.   Two
experiments (9, 39) were vitiated by an accidental cutting of the
spinal cord very far forward, while the fixation was incomplete
and in both of these cases, also, an interesting spirally wound
condition of the fiber was produced.   Apart from these four
experiments, in which there was definite evidence of an interfer-
ence with the condition of the fiber before or after the experi-

[2] Four specimens (1, 25, 27, 31) which died during the progress of the experi-
ment had been so long dead, apparently, as to be worthless for the purpose of
this investigation.   A fifth specimen (33) was unaccountably mislaid.

ment, there are three cases (19, 46, 55) concerning which I am in some doubt as to the correct interpretation of the sections.

Excluding for the present these seven experiments which, for one reason or another, are inconclusive, I have, I believe, very definite evidence concerning the condition of Reissner's fiber in no fewer than forty-six specimens. The conclusions at which I have arrived are based solely upon the reactions in these specimens, about which there appears to be no question.

The subjects of these forty-six experiments may be classified, according to the effect of the experiment upon Reissner's fiber, in four groups.

1. Six specimens (nos. 11, 15, 21, 23, 29, 51) in which it was found that the experimental incision missed the filum terminale and thus failed to break the fiber.

2. Nine specimens (nos. 8, 18, 26, 28, 36, 38, 42, 44, 45) in which the fiber, although broken by the incision, failed to retract forward, or in either direction. The severed end (or ends) were held, apparently, by the adpressed walls of the filum terminale or, in some cases, secured from subsequent slipping by the clotting of blood which had escaped into the central canal from the cut meningeal vessels.

3. This, the largest group, includes thirty specimens in which a more or less extensive retraction of the fiber had followed upon the experimental incision. While in some individuals (37, 52, 53, 64, 66) this retraction was not very great, in others (10, 17, 20, 22, 34, 35, 40, 41, 43, 54, 56, 57, 58, 59, 60, 62, 65, 67, 68) it was very considerable. In at least five (4, 5, 6, 7, 24) it may have been very extensive, also; but, if so, it had been largely repaired before the termination of the experiment.

4. A single specimen (2) in which the process of regeneration was apparently almost completed.

VI. THE RELATION BETWEEN THE CONDITION OF REISSNER'S FIBER AND THE REACTION OBSERVED

1. In the subjects of the experiments

1. Of the six specimens included in the first group two were dogfish. The duration of the experiment varied from a little less than 6 hours (51) to nearly 19 days (23). In not one of these specimens, in which the experimental incision failed to break the fiber was there any reaction.

2. In the second class come nine specimens in which, although the experimental incision was successful in breaking the fiber, this did not undergo retraction forward from the lesion. All but one of these specimens were rays and the duration of the experiment varied from three-quarters of an hour (38) to nearly 7 days (18). For the most part, however, the specimens were killed in the first or second day.

Six specimens were not visibly affected by the operation, while the remaining three exhibited a scarcely perceptible reaction. This took the form either of a very slight uplifting of the tail for a quite brief period (8) or of a trifling elevation of the snout (36, 42).

It would appear, therefore, that a mere breaking of the fiber which is, for any reason, not followed by retraction is unlikely to evoke a reaction and, presumably, does not disorganize the mechanism of which Reissner's fiber forms part.

3. A comparison, however, of the records of the experiments with the evidence afforded by the microscopical examination of the preserved material in the case of the thirty individuals composing the third group, suggests that there exists a distinct connection between the reaction manifested and the retraction of the fiber.

Thus in certain cases (e.g., 37, 52, 53, 64, 66) in which the reaction had not been particularly pronounced or prolonged, there was found to have occurred a comparatively slight retraction. On the other hand, in a number of experiments (10, 20, 22, 24, 35, 43, 54, 56, 57, 58, 59, 60, 67, 68) in which the reaction had been particularly marked there was found to have

occurred an extensive withdrawal of the fiber, forward, from the region of the experimental lesion.

In a couple of instances (47, 64) this relation is less evident, there having been a somewhat pronounced reaction although the fiber had not been very greatly retracted. Both of these specimens were the subjects of experiments of quite short duration ($1\frac{1}{4}$ hours and 10 minutes respectively) and it is probable that the fiber would have continued to retract had the experiments been allowed to proceed for a longer period.

Reissner's fiber, in three specimens (5, 7, 24), all of which were the subjects of experiments of prolonged duration, is found to extend backwards nearly or quite to the region of the experimental incision.

A secondary sinus terminalis is seen in the process of formation in the last of these and in this case Reissner's fiber has become of almost normal diameter but lies freely with a somewhat fibrillated ending in this new terminal enlargement of the central canal.

The sections through the tails of two other specimens were cut transversely and the condition of the end of the fiber can not be certainly determined. In one (7) the fiber has a diameter but slightly greater than the normal, while in the second (5) it is quite distinctly swollen.

Another specimen (6) is apparently a normal case in which there has been a considerable retraction. The terminal piece sectioned shows that the fiber had withdrawn wholly from that region. The penultimate piece, however, contains the free end of the fiber lying slackly and of quite notable slenderness. I suspect that this may be an early phase of regeneration in which a delicate new growth of fiber is stretching backward, the usual simple straightening out of the original thread having, for some reason, been prevented.

4. Regeneration is seen in a well advanced condition in but a single specimen (2), a dogfish, of which the condition of the end of Reissner's fiber is seen in figure 31. In front of the incision, a secondary sinus terminalis has arisen, the pia mater having grown around the end of the filum terminale where it

was severed to form the delicate hinder wall to this new terminal chamber. The fiber seems to have flared out into a terminal plug in which several strands, one somewhat thicker than the normal fiber, can be distinguished. This lies in contact with the meningeal wall of this secondary sinus terminalis and was either just about to become attached to the meninges when the specimen was killed or, more probably, had actually made its new terminal attachment.

5. It will now be convenient to consider more fully the condition of Reissner's fiber in the subjects of eight experiments (3, 9, 19, 39, 46, 49, 55 and 70) which I have refrained from including in either of the four groups, although concerning most of them I have but little doubt as to which category they really belong.

Thus in the case of no. 39 which was an experiment of quite short duration, the sections show that, although broken by the experimental incision, the fiber has not retracted forward from the incision. Since the ray exhibited no reaction after the operation, it is clear that we have a specimen which should be placed in the second of my four groups. During the dissection, however, a slip of the knife inflicted a cut far forward in the spinal cord. As the result of this post-mortem injury, a retraction of the fiber took place from before backwards and a simple spiral twisting has been produced which has affected the fiber back to the region of the incision.

A similar accident occurred to no. 9 with a similar effect upon the fiber. In this case, however, the experiment had been one of considerable duration (11 days) and there had been manifested a well marked reaction. It is extremely probable, therefore, that in this specimen there had resulted the usual considerable retraction of the fiber which had, however, become straightened out before the specimen was killed. Reissner's fiber is found in the sections extending fully to the point where the filum terminale had been severed and, in this resembling the condition of the fiber in no. 39, it is found twisted into a nearly continuous simple spiral (text-fig. 2). There can be little doubt that, but for the accidental breaking of the fiber after the death of the

animal, the experiment would have been found to belong to the third of my four classes.

The case of no. 19 is of a different kind. In this specimen no reaction appeared as the result of the operation yet, in the sections, the severed end of the fiber in front of the lesion was found to be retracted for a short distance, swollen and, near its free end, spirally coiled. The latter detail probably affords the clue to what might, in view of the absence of any reaction, appear as a distinct anomaly. The experiment had continued for 6 days and, therefore, if there had taken place a retraction of the fiber so extensive that the fiber had not straightened out in that time, a well marked reaction should have been evident. Spiral coiling, however, in every other instance known to me, is associated, as I shall show, with recent retraction. In this instance, then, there can be little doubt, I think, that the specimen was one which would in the ordinary way have been included in the second group—i.e., among those in which the fiber was severed but failed to retract—the severed end being gripped, probably, by the compression of the walls of the filum terminale. During the handling which is unavoidable where a rapid dissection is desired, the fiber may have been released and then have commenced to withdraw. A disturbance which freed the fiber from the grip of the walls of the filum terminale doubtless afforded, at the same time, ready ingress to the fixing fluid and thus quickly checked the incipient retraction.

In the last experiment performed (70) there was again an apparent discrepancy. This specimen on recovering from the anaesthetic, assumed a quite unusual position (fig. 10) which appeared to be an obvious reaction to the experiment. Subsequent examination of the material under the microscope revealed, however, that the experimental incision had just failed to cut the filum terminale. As it happens, the greater part of the filum terminale in the piece of tissue sectioned is contained in one thick section in which, although the presence of Reissner's fiber can be ascertained, it is not possible to make out its condition. Owing to a slight distortion of the material, however, the sinus terminalis lies in an adjacent section which is moder-

ately thin. From this terminal region the fiber is certainly absent; the sinus terminalis itself shows signs of disturbance and contains the remains of a clot which is certainly not the result of the experimental incision, which did not destroy the pia mater.

The specimen must have been one which had been brought in recently, probably the previous day, for during my stay at Plymouth I made use of all the moderately small specimens which were available within a very short time of their capture. Since in this specimen, the fiber is absent from the region of the sinus terminalis and the latter chamber itself is somewhat disrupted (for which injury my experiment was not responsible) it is almost certain that the ray had been damaged in the trawl, probably on the previous day.

That there was no reaction in evidence when the specimen was selected for the experiment is doubtless to be explained by the fact that the reaction frequently appears intermittently. Moreover, the experiment was the last undertaken and was performed in some haste so that the usual precaution of keeping the specimen under observation for some hours prior to the operation was not taken in this instance.

The reaction seen in this experiment, therefore, is almost certainly to be attributed to an accidental breaking of the fiber at some time prior to the experiment and is in no way due to the experimental incision which did not disturb the central nervous system.

Two other specimens (3, 49) reveal the fiber broken as the result of some accident, but in these cases the snapping of the fiber must have taken place in the trunk (or head) region and must have occurred only a very short time indeed before the operation.

In the case of the former (no. 3, the subject of the first experiment performed upon a ray) an overdose of chloroform was administered and for some 3 hours following the operation there was no sign of returning animation. At the end of that time it was decided to discontinue the experiment and the central nervous system was exposed and hardened. As it happened, this experiment, while affording no information upon the function

of the fiber, provided material which throws considerable light
upon the recoil of the fiber.

A general account has been given above of the condition of
Reissner's fiber in the hinder part of the spinal cord of this speci-
men. From that description it will be apparent that, since both
before and behind the incision the fiber extends actually to the
severed ends of the filum terminale (text-fig. 4), there could
have been no retraction of the fiber as a sequel to the opera-
tion. Nevertheless, in the condition of the fiber, both in front
and behind the point where it was broken by the operation,
there is very distinct evidence of a recent retraction.

In the terminal (severed) portion of the terminal filament
(text-fig. 4 a) the fiber is seen to be considerably swollen, the
swelling becoming more pronounced in the terminal sinus where
the fiber passes into a loose spiral; the terminal plug is not recog-
nizable, having collapsed, presumably, when the recoil began.

Immediately in front of the incision (text-fig. 4 b) the fiber is
found in a wonderfully twisted state and continues markedly
coiled to the forward end of the piece of tissue examined. The
torsion is not uniform, short simply-twisted stretches interven-
ing between greatly convoluted lengths of fiber. As already
pointed out, this short length of terminal filament contains very
many times its length of Reissner's fiber. It is obvious, then,
that not only must this retraction have been due to a withdrawal
of the fiber from before backwards but, also, that it must have
been in progress prior to the operation, since otherwise it could
not have affected the severed piece of fiber in the region behind
the experimental lesion.

The incipient coiling of the fiber behind the incision is evidence
that retraction had been in progress but for a very short time
when the experimental incision separated this terminal portion
of the fiber and, as it happened, checked further recoil behind
this point. In front of the lesion, however, a gradual retrac-
tion continued during the 3 hours while the specimen lay mo-
tionless,[3] until a great length of Reissner's fiber had accumu-
lated in the hinder part of the spinal cord.

[3] Doubtless the retraction actually continued until finally stopped by the
hardening action of the fixing fluid.

That the fiber was broken just prior to the experiment, in no. 49, also, appears extremely probable. In the trunk region the central canal of the spinal cord is found empty of fiber, while in the hinder part of the spinal cord and the filum terminale the fiber is seen much coiled and swollen throughout the entire length of two pieces of the tail region which were sectioned. Here, too, as in no. 3, there was practically no retraction forward from the lesion (fig. 22) so that the whole of the retraction observed must have resulted from the backward withdrawal of the fiber towards the tail. Behind the incision the fiber extends to the severed end of the terminal filament but is loose and undulating, shows some spiral winding and, near the terminal sinus, is distinctly swollen. As in the previous case, therefore, we have the evidence of a retraction which has started prior to the operation and was the result of an accidental breakage of the fiber far forward in the trunk region. In this case the specimen recovered from the anaesthetic and manifested a marked reaction, not to be attributed to the experimental incision. The experiment, however, was of much shorter duration than was the case in no. 3 and the less intricately coiled condition of the fiber in this specimen is clearly related to the shorter period during which the fiber was free to withdraw. In nos. 9 and 39, in which the accidental cutting of the fiber took place after death, the fiber was free to retract only for the much shorter period which was required for the penetration of the fixing fluids. In both of these specimens the fiber has simply undergone a fairly regular twisting but has not produced the more complicated secondary spirals seen in no. 49 and, still better developed, in no. 3.

In both of the two experiments which remain to be considered (nos. 46 and 55) the fiber appears as an extraordinary delicate filament lying somewhat slackly but not apparently withdrawn from the injured place. In neither case was the experiment of long duration and in both the reaction observed took on a somewhat unusual character, there being manifested a distinct departure from the normal pose but no appreciable deviation of the long axis from the regular straight line. While this reaction

was not one which I should be inclined to describe as 'marked' it was, nevertheless, too considerable to be attributed to the scarcely appreciable retraction which has occurred at the severed end of the fiber.

If, then, I am correct in regarding the occurrence of this exceptionally delicate fiber as indicative of an early stage in a new backward growth of the fiber after some unusually extensive retraction, the reaction noticed in these two experiments may perhaps have been the consequence of a renewed disturbance of the Reissner's fiber mechanism in specimens in which the repair of a previous disturbance had scarcely been completed.

The condition of Reissner's fiber in the subjects of these eight experiments may therefore be summed up as follows.

One (19) is to be regarded as exhibiting a slight retraction of the fiber started at the moment of fixation of the material and quickly checked; two others (9, 39) showed a considerable retraction resulting from an accidental cutting of the fiber during the dissection made to expose the central nervous system. The remainder are regarded as showing stages in the retraction (or repair) of the fiber consequent upon a breaking of the fiber prior to the experiment. This snapping of the fiber may have occurred immediately (3, 49) or some little time (70) or some considerable time (46, 55) before the incision was made. That such a breakage of the fiber does occur not infrequently in life and that it may produce a reaction comparable to that induced by artificial section of the fiber will be seen from the account given in the following section.

2. Non-experimental material

An attitude similar to that induced in many specimens by the experimental incision, was occasionally noticed in specimens (not the subjects of the experiments) confined in the aquarium of the Plymouth Biological Station.

Of these, one—a dogfish (F)—was obtained during the summer of 1910. It had been seen in the aquarium at intervals extending over several days with both head and tail well up.

Finally, it was captured and a close scrutiny revealed a slight external injury to the hinder margin of the caudal fin, which had a frayed appearance and from which a narrow strip of tissue (including the extremity of the filum terminale) had been scraped away. In sections subsequently prepared, it was seen that the sinus terminalis and the hinder end of the terminal filament were wanting. Reissner's fiber had evidently been broken and had, doubtless, undergone a very considerable retraction, but at the time the material was preserved the fiber had returned almost to normal size and stretched backwards to the damaged end of the terminal filament. For the most part, it lay in a fairly even course but near the actual end it was slack and lay in gentle undulations. The central canal apparently opened freely to the exterior and there were no signs of the formation of a new secondary sinus terminalis.

During the following summer, several specimens (both rays and dogfish) exhibiting the reaction (of an abnormal attitude in repose) which is associated with the broken and retracted condition of the fiber were taken from the tanks of the aquarium.

A piece of the tail (including the terminal portion of the central nervous system) of several of these specimens and, in some cases, a piece also of the spinal cord, or the brain, were sectioned.

The first of these (Raia XIX) was brought in on August 2, the tail showing numerous abrasions obviously received in the trawl. The specimen was isolated and next morning was found showing a well marked reaction. Later that same day (about 24 hours after its capture) it was killed and the nervous system exposed and preserved in the usual manner.

In the sections, the terminal filament and sinus terminalis are found apparently undamaged but Reissner's fiber had certainly been broken, at or near the sinus terminalis. The free (broken) hinder end of the fiber was found near the sinus terminalis—almost brush-like (fig. 19). Traced forward from this point, the fiber is seen slack, swollen and snarled (fig. 25). It emerges from the anterior end of the tangle in a loosely coiled spiral, the twisted portion passing into a straight stretch

which continues much swollen to the forward end of the piece of tissue sectioned.

In a second specimen of the same species showing the characteristic reaction (*Raia clavata*, XXXIX), the free end of the broken fiber was found near the sinus terminalis swollen and loosely spirally coiled (fig. 20). In this case I have no note as to the date of the capture. Almost certainly, however, the specimen was one of a batch of small rays (of which others were 38, 39, 40) which had been brought in on the previous day. As in the previous specimen, the end of the central nervous system appeared quite uninjured (apart from Reissner's fiber) and in this case there were no signs of external injury.

In two other rays showing the reaction (XXXV and LXXIII) there was evidence that the fiber had been broken for, in both, the fiber was found lying slackly in the region examined (the filum terminale) and in the case of the former it was distinctly swollen, but the free end was not found in my sections.

Another ray (XXXIII) and a dogfish (P), although manifesting the usual reaction, retained a normal condition of the fiber in the tail region, of which alone sections were examined. It is seen stretching apparently tensely and of normal size and it is probable that the fiber must have been broken in a region too remote to cause a disturbance of the fiber in the terminal filament.

VII. DISCUSSION

1. The function and mode of action of the Reissner's fiber apparatus

In an earlier paper ('12, p. 429) I showed that the breaking of the fiber in life generally resulted in the recoil of the severed ends, and I concluded that the effect of such a breakage was to bring about a temporary loss of control over the pose of the body when at rest (and probably also whilst in motion).

Where the fiber is broken from natural or accidental causes it appears that retraction of the broken end almost invariably ensues, but in the subjects of the experiments this retraction may be, for a while, delayed or even prevented altogether. It

was pointed out that in the single example (8) in which the fiber, although broken, had failed to retract there had been no marked reaction.

These conclusions, based upon the examination of material from a comparatively small number of experiments, are strongly supported by the results of the much more numerous experiments which were subsequently performed. The examination of the condition of Reissner's fiber in several specimens which were not the subjects of experiment has provided further evidence in corroboration of the correctness of those conclusions.

The results of this investigation may be said, therefore, to afford very definite confirmation of Dendy's suggestion (put forward in 1909) that the fiber forms part of a mechanism which is concerned in the automatic regulation of the flexure of the body. This hypothesis is quite in harmony, moreover, with certain observations recorded by Sargent ('04), although that author interpreted the facts in an altogether different sense (vide infra).

I have been unable, however, to determine whether the reaction (the assumption of an unnatural attitude at rest and an abnormal action whilst in motion) is to be regarded as the consequence of the diminution of tension at the sub-commissural organ due to the slackening of the fiber or whether it is to be attributed to the putting out of action of a larger or smaller number of the scattered sensory cells situated in the epithelium of the central canal.

It has been pointed out that, although there has been found, in some cases, a quite considerable retraction of the fiber in the hinder part of the spinal cord, accompanied by much swelling and spiral winding, yet in the anterior region of the spinal cord and in the brain itself the fiber may appear to be practically normal. In such a case it seems improbable that any appreciable diminution in the tension of the fiber could have been felt in the region of the sub-commissural organ. Further, in those examples in which the slackness of the fiber has extended far forward, even though it be not accompanied by swelling, it is inconceivable that it could have taken place without rupturing a

great number of those delicate component fibrillae (as I believe them to be) which serve to support and stay the fiber along the length of the spinal cord.

In the subject of two of the experiments (3, 49) the fiber had broken very far forward; of these, one failed to recover consciousness and gave no reaction, but it is extremely significant that in the other (49) the reaction took on a somewhat peculiar form. It is suggested, therefore, that in this case (where the breaking of the fiber must certainly have reacted upon the subcommissural organ), the more pronounced reaction was the sequel of an unusually extensive disorganization of the apparatus.

In this connection, it is interesting to recall what has been recorded by Sargent concerning his experiments. That author laid much stress upon the fact that the subjects of his experiments would blunder, headlong, into obstacles (stationary or other). This behavior, as I have already pointed out ('12, p. 420), is to be noted in the lesser dogfish both in normal (control) specimens as well as in the subjects of the experiments when removed from the comparatively spacious tanks of the aquarium to the smaller tanks in which, alone, one can be certain of keeping them under close observation. Moreover, this blundering gait disappeared, after a few days confinement in the more limited space, in the subjects of the experiments as well as in the control specimens.

In the larger sharks of which Sargent made use, and which were apparently freshly caught specimens, one cannot wonder at such a result. Moreover, Sargent had no opportunity to observe the passing of this phase, for his specimens after a day or so became quite lethargic and died upon the fourth or fifth day of the experiment. Nor does the failure of the fish to avoid collision with the walls of its cage bear out Sargent's contention that there was in these specimens, a delay in the transmission of the optical stimulus, for such an object, always present, would be visible for a sufficiently long period to allow any optical stimulus to pass by the ordinary conduction paths. Even where an obstacle might be interposed with extreme suddenness it would have been scarcely possible to observe any delay

in the 'avoidance reaction,' for Sargent's calculations apparently suggest that, in an animal a metre in length, the passage of a stimulus along the conduction path alleged to be provided by Reissner's fiber might effect a saving of one-fiftieth of a second, at most.

I believe myself that there is nothing in this behavior but what may reasonably be attributed to excitement due to the handling inevitable to the change of accomodation, the strangeness of the new environment and the frantic attempt to escape from the more cramped enclosure. It is interesting, therefore, to find that in one specimen, described by Sargent as probably abnormal because it had been in confinement for a considerable period (and was therefore accustomed to its enclosure) this blundering into stationary and movable obstacles (the 'slow optical response') was not observed.

As already remarked, this heedlessness in movement disappeared in my specimens (control and experimental) within a few days when the fish had become accustomed, presumably, to its new surroundings. In the case of the subjects of Sargent's experiments (in which the septic condition set up in the brain, by the operation, brought about a marked lethargy and speedy death) there was insufficient time for the specimens to become habituated to an alteration in their environment.

While, therefore, certain of the phenomena observed by Sargent are, in my opinion, to be attributed merely to a change in the external condition of his specimens or to the ill-effects of the operation upon the entire organism, others of the reactions, upon which Sargent has laid little stress, may very well, I think, have been a consequence of the breaking of the fiber. Sargent noted that the fish adopted most abnormal attitudes in swimming, actually turning even and swimming (not floating) ventral surface uppermost. One specimen is described as swimming 'with its head curved dorsally' a reaction clearly suggesting a loss of control of the posture. In none of my experiments was there manifested so marked a reaction, but in none of my experiments was the fiber cut so near to the subcommissural organ (in the fourth ventricle).

The less noticeable reaction which I have recorded of so many of my specimens (viz.: the departure of the long axis of the body from the normal position in repose) was very probably exhibited by Sargent's specimens, also, when at rest. This, however, would be little likely to attract the attention of an observer who was viewing the specimens (whether confined in the cage or free in the pool) from above as they must of necessity have been viewed.

Such evidence, then, as is available suggests that the nearer the break in the fiber is to the sub-commissural organ, the more pronounced is the reaction. On the other hand, there is a marked reaction in many cases in which there has been no apparent interference with the taut condition of the fiber in the anterior part of the spinal cord and brain and in which, therefore, it might be supposed that the sub-commissural organ is little if at all affected.

While, therefore, I am inclined to agree with Tretjakoff ('13) in assigning a considerable importance to the detached sensory cells distributed along the entire length of the central canal (and possibly also in the isthmic canal), I think that there can be little doubt concerning the supreme importance of the sub-commissural organ as the center of this sensory apparatus.

That Dendy's suggestion of the manner in which the stimulus may be supposed to be brought to bear upon its related sensory cells by alterations in the tension of the fiber is much more probable than Tretjakoff's view that the stimulus is a result of pressure of the fiber upon sensory cells admits in my mind, of little doubt. As already pointed out, I believe that Tretjakoff's statements upon this point are based upon a study of material in which a retraction of the fiber had taken place and in which, therefore, the normal anatomical relations of the fiber are not seen. I interpret the 'knobbed ends' of the sensory processes as the remains of the broken fibrillae (which had connected Reissner's fiber with the sensory cells in the ependymal epithelium of the canalis centralis) retracted to the parent cells.

2. *The spiral winding of the fiber and the occurrence of 'snarls'*

Examples of the peculiar spiral contraction of the fiber have been seen in a number of the experiments. Thus numbers 3, 4, 9, 19, 34, 35, 37, 41, 49 and 56 all show the fiber twisted to a greater or less extent. That the list is not more lengthy is to be explained by the fact that in a number of cases I have not cut sections of the spinal cord sufficiently far forward to find the retracted end.

There is distinct evidence that, in the case of no. 3, this retraction (though not the result of the experimental incision) must have taken place, for the most part, during the three hours or so which elapsed between the operation and the fixation of the material. The similar but less extensive coiling which occurred in no. 49 (again not the result of the experimental incision) must, likewise, have been produced almost wholly after the fiber was severed by the operation, for the severed portion of the fiber behind the incision is but little affected. This specimen was allowed to live but an hour and a half after the beginning of the experiment and there is doubtless a connection between the less intricately coiled condition of the fiber in this specimen and the shorter period which elapsed between the breaking of the fiber and fixation.

The case of no. 9 differs from that of the two preceding specimens in that the cut which started the backward recoil was made after the death of the specimen just as the material was about to be plunged into the fixing fluid. The simple and continuous spiral winding which is found in this specimen can have been produced, therefore, only during the time which was necessary for the fixing fluid to thoroughly penetrate and harden the material.

In the case of experiments 34 and 56 (both of which were of short duration) the fiber has retracted (spirally) away from the lesion and the recoil, therefore, was definitely a consequence of the experimental incision. The character of the spiral winding in the fiber in the case of four other experiments (4, 35, 37, 41) is somewhat different. It is found at the free end but does not

extend for a great distance along the fiber and has a much looser twist which suggests the uncoiling of a spirally twisted thread. All four of these experiments had a relatively considerable duration, the subject being killed towards the end of the first day or during the second.

Apart from experiment 9 which was vitiated subsequently (by an accidental cut during dissection), the only case in which even a slight spiral twisting was observed in an experiment of long duration is no. 19. The subject was a ray, which, during the whole time (nearly 7 days) which elapsed between the breaking of the fiber and the killing of the specimen, gave absolutely no reaction. I have already suggested that it is probable we are dealing, in this case, with a slipping and coiling of the fiber which was started during the dissection by a reopening of the wound made by the operation but was quickly checked by the rapid penetration of the fixing fluid.

With but this single possible exception, therefore, every case of spiral winding of the fiber has been found in the early stages of the experiment or immediately following an accidental cut; by the second day this torsion has usually disappeared and, where it is found at so late a period, is almost certainly in process of uncoiling.

In confirmation of the view that the spiral coiling is found as the result of a comparatively recent snapping of the fiber, it may be noted that, while it has been found frequently in material in which the spinal cord has been severed during or immediately prior to fixation, it is more rarely found in specimens of which the nervous system had been preserved entire.

In many larval lampreys and in adult myxinoids, all of which were preserved entire, I found, it is true, an intricately coiled mass of fiber which, in some cases, almost fills the sinus terminalis[4] ('12 a, figs. 15, 17, 18). In none of these specimens had there been any attempt to expose the central nervous system

---

[4] That this terminal coiled mass, though so frequently found, is not, as Studnička ('99) supposed, the normal condition is proved by the fact that Sanders has seen a taut condition of the fiber in the sinus terminalis ('94, p. 11) comparable to that which I have described in the lamprey and other forms ('12, '12 a).

and fixation, therefore, had necessarily been slow. I have suggested ('12 a, p. 27) that unusually strenuous exertions made by the animals in their unavailing efforts to avoid capture may be the cause of the somewhat frequent instances of broken fiber noted in these cyclostomes.[5] Careless handling of the specimens might be equally responsible for the snapping of the fiber. It is possible, therefore, that the apparent absence of the fiber from the spinal cord of specimens which have been some time dead before fixation may not be due (as I have supposed) simply to the degeneration of the fiber having already occurred, but may be owing rather to its having been broken and retracted entirely beyond the limits of the piece or pieces of tissue examined.

In two rays which, although not the subjects of experiments, manifested a well marked reaction a spirally coiled condition was discovered in the broken fiber. One (Raia XIX) is known certainly to have been taken some 24 hours before the material was preserved and it is probable that a similar interval had elapsed between the capture and preservation of the material in the case of the second (XXXIX) also. The former bore signs of recent damage in the tail region evidently caused by the trawl but the other appeared externally to be undamaged. If, therefore, the fiber had been broken at the time of capture, in consequence of the violence of the animal's struggles to escape, the fiber might be expected to have become straightened out or to be in the process of unwinding. In one (XXXIX) the free end of the fiber is loosely curled (fig. 20) and in the other (XIX) a tangle remains as evidence of a sharp recoil, but the spiral winding is found only in the vicinity of the tangle (fig. 25) and has, elsewhere, disappeared.

That the fiber was liable, in its recoil, to form intricately tangled knots or 'snarls' was first noticed by Sargent ('04, fig. 8). His figure does not suggest the spiral winding which I believe to be associated with the recent contraction of the fiber and which is seen in the photomicrograph which I published in 1912 ('12, fig. 3).

---

[5] The breaking of the fiber, prior to the experiment, in nos. 3 and 49 is probably to be attributed to this cause.

At the commencement of this investigation I inclined to the idea that such a knot would be invariably produced when the broken fiber retracted, and supposed that the spiral winding, extending more or less uniformly along a great length, would be found only in those cases where a gradual process of fixation prevented the more sudden recoil. The results obtained from a large number of experiments indicate, however, that this sudden contraction may be of much less general occurrence than was supposed and that the withdrawal of the fiber is brought about usually, by the simple spiral twisting of the fiber.

The tightly knotted tangle of fiber present in Raia XIX, just above mentioned, is the only example of this condition which I have encountered in the course of this investigation. There is reason to believe that in this case the fiber may have broken some 24 hours before the material was preserved. In another specimen (10) in which the fiber was broken by the experimental incision made some 10 days before the fixation of the material, there is found a loosely twisted skein of fiber, a small part of which is represented in figure 26. That this condition had been preceded by the tightly knotted condition is very probable, this knot having doubtless served to hold the broken end and thus prevent more extensive retraction, for the tangle is found at no great distance (about half an inch) from the point of injury. It must be supposed, therefore, that during the 10 days of the experiment the spiral torsion had disappeared and the process of disentangling the snarl had been proceeding. The fiber, except at its immediate hinder end, had become nearly normal in size, but whether the tangle would have been smoothed out eventually, if the experiment had been prolonged, or whether a new delicate growth from the hinder free end would have followed, I have no evidence to decide.

3. The duration of the reaction and the problem of regeneration

It is not quite obvious why the reaction appears to be so variable in its duration. If the assumption of an abnormal attitude in repose is, as I believe, a consequence of the disorganization of the mechanism of which Reissner's fiber forms part, we

should expect that the reaction would continue until the fiber had reëstablished its attachment to the walls of the sinus terminalis and had once more attained to its normal tension.

The reaction did persist, indeed, in some specimens until the attachment was practically made good (2) or until the termination of the experiment (9). Occasionally the reaction was manifested intermittently for several days (5, 6) while in one case (24) it reappeared after several days of apparent normality. On the other hand, a reaction was sometimes marked during the early hours of an experiment but was not noticed subsequently (7) although the sections showed that the fiber had been broken but gave no indication that the new terminal attachment was completed.

While, then, it is quite possible that in some specimens (in which the reaction had seemingly disappeared and which were killed very soon after the operation) the reaction might have reappeared at intervals had the experiment been prolonged, it is probable that in many the reaction would have apparently completely vanished (as in 7).

Nevertheless, it seems unlikely that the effect produced by the breaking and retraction of the fiber can really altogether disappear until the tension of the fiber has been restored.

The more obvious irregularities of the pose are possibly soon corrected, to a large extent, by the aid of the other senses, notably that of touch, and these corrections would be likely to become more exact as time passed, thus accounting, in some measure, for the gradual diminution in the magnitude of the reaction. It is, however, extremely probable that there may have been other reactions which persisted long after the specimens seemed to me to be normal. Especially may this have been the case with minute irregularities in action, in swimming, for whilst in motion the correcting influence of the sense of touch, at least, would almost certainly be eliminated. The motion of the animal is particularly difficult to observe closely and defied my attempts at analysis so that, although at times I felt convinced that the action was not exactly that which is

usual, yet it was extremely difficult to decide wherein the difference lay.

There is yet another possible explanation. It has been seen that, where retraction did not follow the breakage of the fiber, there was no obvious reaction. It has been assumed that this was due to the maintenance of the tension of the fiber by the firm grip of the adpressed walls of the filum terminale upon the severed end of the fiber. Not only, however, might the tension be maintained but the connections with the numerous sensory cells in the central canal, whose filamentous processes contribute to the substance of the fiber, are preserved intact. The absence of the reaction may be partly or wholly attributable to this latter fact for, although the terminal plug at the hinder end of the fiber is clearly to be regarded as the principal insertion of the fiber, yet there can be little doubt that the attachments of the fiber by the component fibrillae throughout the length of the spinal cord must afford a very considerable support. Such evidence as these experiments have afforded suggests that the greater the extent of the retraction forward the greater is the degree of the reaction. It may well be, then, that as soon as the forward retraction is checked and the repairing process has brought about the unwinding and straightening out of the fiber, the component fibrillae may forthwith begin to renew their attachment to the fiber. In this way while they may assist in restoring the tense condition of the whole fiber a constantly increasing number of sensory cells may be coming into action again, the diminution of the visible reaction being attributable to the restoration of these connections.

Sargent has stated ('04, p. 230) that "sharks have shown almost no capacity to heal wounds or regenerate skin." During the progress of this investigation several rays were taken in which there was evidence of the loss of part of the tail but the stump had healed perfectly. While it may be that, as regards this power of regeneration, rays differ from sharks and dogfish, it must be remembered that my rays were, in general, quite small specimens and it is exceedingly likely that the injury had been inflicted when the specimens were very small, indeed.

In young animals the recuperative powers are frequently much
greater than in aged specimens and it may prove that the
restoration of the normal (functional) condition of Reissner's
fiber after injury may be effected much more quickly in some
specimens than in others.

In this connection the condition of Reissner's fiber in the
dogfish (F) is of interest. This specimen, it will be remembered,
was one which was seen in the large aquarium tank exhibiting,
very markedly, the reaction which is associated with the broken
and retracted fiber. The fish had certainly been in confine-
ment for some time and there was reason for connecting the
injury to the hinder border of the caudal fin with damage in-
flicted by the trawl. The injury was, therefore, probably of
long standing and the reaction had almost certainly persisted
for a considerable time. In the sections the fiber was found to
be somewhat slack, lying near its free end in loose undulations
and there were no indications that regenerative processes were
at work. The specimen was unusually large and presumably
an old individual. There seems, therefore, to be in this case
a connection between the size (age) of the specimen, the lack of
regenerative powers and the continuance of the reaction.

As already noted, I have but one example of undoubted re-
pair of the mechanism (fig. 31) after the experiment. This is
seen in a dogfish (2) which was killed sixteen days after the
operation.

In several cases, however, the fiber had clearly undergone a
considerable retraction but had straightened out again before
the specimens were killed. In the sections, therefore, it is seen
to extend almost or quite to the spot where it had been broken
by the experimental incision. Examples of this phase of repair
are to be seen in nos. 5, 7, 8 and 24. The disentanglement
of the snarl (10) and the unwinding of the spirally twisted
fiber (37, 41) must be regarded as preliminary stages in the proc-
ess of repair. In no. 18, where there had been no retraction,
it would seem as though the end of the fiber was flaring out in
preparation for a new terminal plug (fig. 28).

Whether the delicate fiber found extending backward slackly in some specimens (46, 55) almost to the region of the incision, is to be regarded as yet another early stage in regeneration is less certain.   Possibly it is a backward growth from the free end of the fiber in a snarl which has altogether failed to become disentangled.   On the other hand the fineness of the fiber may be nothing but an individual peculiarity.

It is noteworthy that, where this abnormally fine fiber was found, it had retracted but little when cut and the consequent reaction had proved to be but slight.

In view of the fact that a case of complete repair had been obtained in but a single experiment (2), it has not been possible to determine the period within which regeneration might normally be expected to take place.   If the incision has completely divided the filum terminale (in practice an inevitable consequence of any attempt to cut the fiber) even though this be very far posteriorly, it is almost certain that regeneration cannot be effected until a new (secondary) sinus terminalis has been formed. It is unlikely that this comes into existence earlier than the end of the second week, although the exact time would depend upon the regenerative powers of the tissue of the individual and would be likely to take place more quickly in young and rapidly growing specimens.   The new attachment of the fiber may, however, be even then delayed if the retraction has been very considerable, has resulted in a tangled knot, or if the fiber has broken very far forward.

In several of my experiments the fiber had returned nearly to its normal diameter and had pushed backwards to the region of the incision within a week of the operation, while the spiral twist appears to be straightened out during the second day under ordinary circumstances.   A complicated tangle evidently requires a considerable period in which to become resolved and in such a knot it is probable that the spiral twisting may persist rather longer.   In my experiments, however, only a short length of the fiber was actually separated and consequently no great length of new growth, if any, was required to enable the fiber to extend to the newly formed sinus terminalis.   Where,

however, the fiber may have been broken by an accident at a point very far forward it may be supposed that a relatively long period may elapse before the new growth has pushed back to the terminal sinus.

But regeneration might, under such circumstances, take place much more quickly when (unlike the condition after the experiment) the walls of the central nervous system had remained intact and there was no need for the production of a new terminal sinus.

Specimens 38 and 41 are instances in which regeneration must certainly have occurred, both of these rays having suffered the loss of the hinder part of the tail (and therefore of the sinus terminalis) earlier in life.

VIII. SUMMARY

1. Reissner's fiber, if severed, will generally be withdrawn in both directions from the lesion, the retraction being apparently effected by a spiral winding of the fiber which attains a greatly increased thickness as the withdrawal proceeds.

2. In dead or dying material, this retraction may continue, if not checked by prompt fixation, until the whole of the fiber has withdrawn to its points of attachments; in living specimens there may be produced at the broken ends a tangle or snarl which doubtless serves to prevent such extensive retraction.

3. In individual rays or dogfish in which such retraction of the fiber has taken place there is manifested a distinctive reaction; the specimen assumes an abnormal posture while at rest and probably, also, exhibits an unusual action while in motion.

4. This reaction becomes apparent very shortly after the return to consciousness (of the animal anaesthetized for the operation), may be intermittent, and is manifested by different specimens for widely different periods. Probably there is a connection between the degree of the reaction and the extent of the retraction of Reissner's fiber.

5. This reaction is not observed in those individuals in which the fiber has been broken but has, for any reason, failed to retract.

6. The time required for regeneration is probably not less than a week, even when the retraction has not been extensive and the filum terminale and sinus terminalis are undamaged; in the case of the experimental material probably several weeks are required. Regeneration commences with the uncoiling of the fiber, which may be complete in a couple of days. The fiber extends backwards more or less slackly, becomes less swollen and probably by further growth, comes once more into contact with the hinder wall of the sinus terminalis (original or secondary) into which it becomes inserted.

7. It would appear, therefore, that, as suggested by Dendy, the fiber serves to control automatically the flexure and pose of the body. While it is probable that the related sensory cells are largely concentrated in the sub-commissural organ, it is equally probable that many other such sensory cells are scattered in the ependymal epithelium of the canalis centralis throughout the length of the spinal cord.

IX. LITERATURE CITED

ALLEN, W. F. 1916 Studies on the spinal cord and medulla of cyclostomes with special reference to the formation and expansion of the roof plate and the flattening of the spinal cord. Jour. Comp. Neur., vol. 26.

AYERS, H. 1908 The ventricular fibers in the brain of myxinoids. Anat. Anz., Bd. 32.

BEARD, J. 1896 The history of a transient nervous apparatus in certain Ichthyopsida. Zool. Jahrb., Bd. 9.

DENDY, A. 1902 On a pair of ciliated grooves in the brain of the ammocoete, apparently serving to promote the circulation of the fluid in the brain cavity. Proc. Roy. Soc., vol. 69.
1909 The function of Reissner's fiber and the ependymal groove. Nature, vol. 82.
1912 Reissner's fiber and the sub-commissural organ in the vertebrate brain. Brit. Assoc. Adv. Sc., 1912.

DENDY AND NICHOLLS 1910 On the occurrence of a mesocoelic recess in the human brain and its relation to the sub-commissural organ of lower vertebrates, with special reference to the distribution of Reissner's fiber in the vertebrate series and its possible function. Proc. Roy. Soc., Lond., vol. 82.

EDINGER, L. 1892 Untersuchungen über die vergleichende Anatomie des Gehirnes. Abhandl. Senck. Naturf. Gesells. Frankt., Bd. 18.
1908 Vorlesungen über den Bau der nervösen Centralorgane des Menschen und der Tiere. Vergl. Anat. des. Gehirnes, Bd. 2, 7 Aufl., Leipzig, 1908.

GADOW, H. 1891 Vögel., Bronn's Klassen und Ordnungen des Thierreichs, Bd. 6, Abth. 4.

HORSLEY, V. 1908 Note on the existence of Reissner's fiber in higher vertebrates. Brain, vol. 31.

HOUSER, G. L. 1901 The neurons and supporting elements of the brain of a selachian. Jour. Comp. Neur., vol. 11.

KALBERLAH, F. 1900 Über das Rückenmark der Plagiostomen. Ein Beitrag zur vergleichenden Anatomie des Centralnervensystems. Zeitschr. f. ges. Naturwiss., Bd. 73.

KÖLLIKER, A. 1902 Über die oberflächlichen Nervenkerne im Marke der Vögel und Reptilien. Zeitschr. f. wiss. Zool., Bd. 72.

KOLMER, W. 1905 Zur Kenntniss des Rückenmarkes von Ammocoetes. Anat. Hft., Bd. 29, Abth. 1.

KUTSCHIN, O. 1863 Über den Bau des Rückenmarks von Neunauges. Kasan, 1863. Abstract by Stieda, Arch. mikr. Anat., Bd. 2, 1866.

NICHOLLS, G. E. 1909 The function of Reissner's fiber and the ependymal groove. Nature, vol. 82.
1910 See DENDY AND NICHOLLS.
1912 An experimental investigation on the function of Reissner's fiber. Anat. Anz., Bd. 40.
1912 a The structure and development of Reissner's fiber and the sub-commissural organ. Quart. Journ. Micr. Sc., vol. 58.

Nicholls, G. E. 1913 An experimental investigation on the function of Reissner's fiber. Jour. Marine Biol. Assoc., vol. 9.

Reissner, E. 1860 Beiträge zur Kenntniss vom Bau des Rückenmarks von Petromyzon fluviatilis. Arch. f. Anat. u. Physiol., Jahrg. 1860.

Rohon, J. V. 1877 Das Centralorgan des Nervensystems der Selachier. Denks. Akad. Wiss. Wien. Math. naturw. Kl., Bd. 38.

Sanders, A. 1878 Contributions to the anatomy of the central nervous system in vertebrate animals. Ichthyopsida. Pisces. Teleostei. Phil. Trans. Roy. Soc., Lond., vol. 169.
1894. Researches in the nervous system of Myxine glutinosa. London.

Sargent, P. E. 1900 Reissner's fiber in the canalis centralis of vertebrates. Anat. Anz., Bd. 17.
1901 The development and function of Reissner's fiber and its cellular connections: a preliminary paper. Proc. Amer. Acad. Arts and Sc., vol. 36.
1903 The ependymal grooves in the roof of the diencephalon of vertebrates. Science, n.s., vol. 17.
1904 The optic reflex apparatus for short-circuit transmission of motor reflexes through Reissner's fiber; its morphology, ontogeny, phylogeny and function. Part I, The fish-like vertebrates. Bull. Mus. Comp. Zool. Harvard.

Stieda, L. 1868 Studien über das centrale Nervensystem der Wirbelthiere. Zeits. f. wiss. Zool., Bd. 19.
1873 Über den Bau des Rückenmarkes der Rochen und der Haie. Zeits. f. wiss. Zool., Bd. 23.

Streeter, G. L. 1902 The structure of the spinal cord of the ostrich. Am. Jour. Anat., vol. 3.

Studnička, F. K. 1899 Der Reissner'schen Faden aus dem Centralkanal des Rückenmarks und sein Verhalten in dem Ventriculus (Sinus) terminalis. Sitzb. k. bohm. Ges. Wiss. math. nat. Kl., Prag.
1900 Untersuchungen über den Bau des Ependyms der nervosen Centralorgane. Anat. Hft., Abth. I, Bd. 15.
1913 Das Extracellulare Protoplasma. Anat. Anz., Bd. 44.

Tretjakoff, D. 1909 Das Nervensystem von Ammocoetes. I. Das Rückenmark. Arch. f. mikr. Anat., Bd. 73.
1913 Die zentralen Sinnesorgane bei Petromyzon. Arch. f. mikr. Anat., Bd. 83.

Viault, F. 1876 Recherches histologiques sur la Structure des Centres nerveux des Plagiostomes. Arch. Zool. expér. gen., Tom. 5.

EXPLANATION OF FIGURES

1 Scyllium canicula. A photograph of a normal specimen upon which no experiment had been performed. The lower border of the caudal fin is seen lightly resting upon the floor of the tank.

2 A photograph of the subject of experiment 22, taken nearly five hours after the operation, showing a moderate reaction. *, indicates the region of the incision.

3 A photograph of the same dogfish, taken half an hour later, the tail being rather more lifted.

4 A photograph of the subject of experiment 34, taken about two hours after the operation, showing a well marked reaction.

5 A photograph of the subject of experiment 24, taken upon the third day of the experiment, and showing a well marked reaction of the tail.

6 A photograph of the subject of experiment 23, the specimen being seen reposing in the attitude adopted by normal dogfish. The photograph was taken upon the fourth day of the experiment, no reaction having appeared.

7   Photograph of a normal ray (not the subject of an experiment), showing in side view, the attitude of repose which is normal in these animals.

8   A photograph of the subject of experiment 31, showing a reaction affecting the tail only.   *, indicates the region of the incision.

9   A photograph of the subject of experiment 68, taken about two hours after the operation.   (Cf. figure 13, a photograph of the same specimen taken half an hour earlier.)

10   A photograph of a normal ray (not the subject of an experiment), showing the natural position of the snout in the ray when at rest.

11   A photograph, taken several hours after the operation, of the subject of experiment 30.   This ray was one which, while it occasionally showed the typical reaction, rested for the most part in the attitude shown.

12   A photograph, taken a quarter of an hour after the operation, of the subject of experiment 70.   The head is seen well raised but the tail appears unaffected.

13   A photograph of the subject of experiment 68, taken an hour and a half after the operation.   The tail is seen, somewhat indistinctly, well raised and turned to the left.

14   A photograph of the subject of experiment 56, taken half an hour after the operation, showing an extreme reaction, the body being raised completely from the floor and supported only upon the lateral border of the pectoral fins.

15   A photograph of a ray (XXXIII), not the subject of an experiment, but in the attitude which results from the breaking and retraction of Reissner's fiber.   This photograph was taken after the ray had been kept for four days under observation.
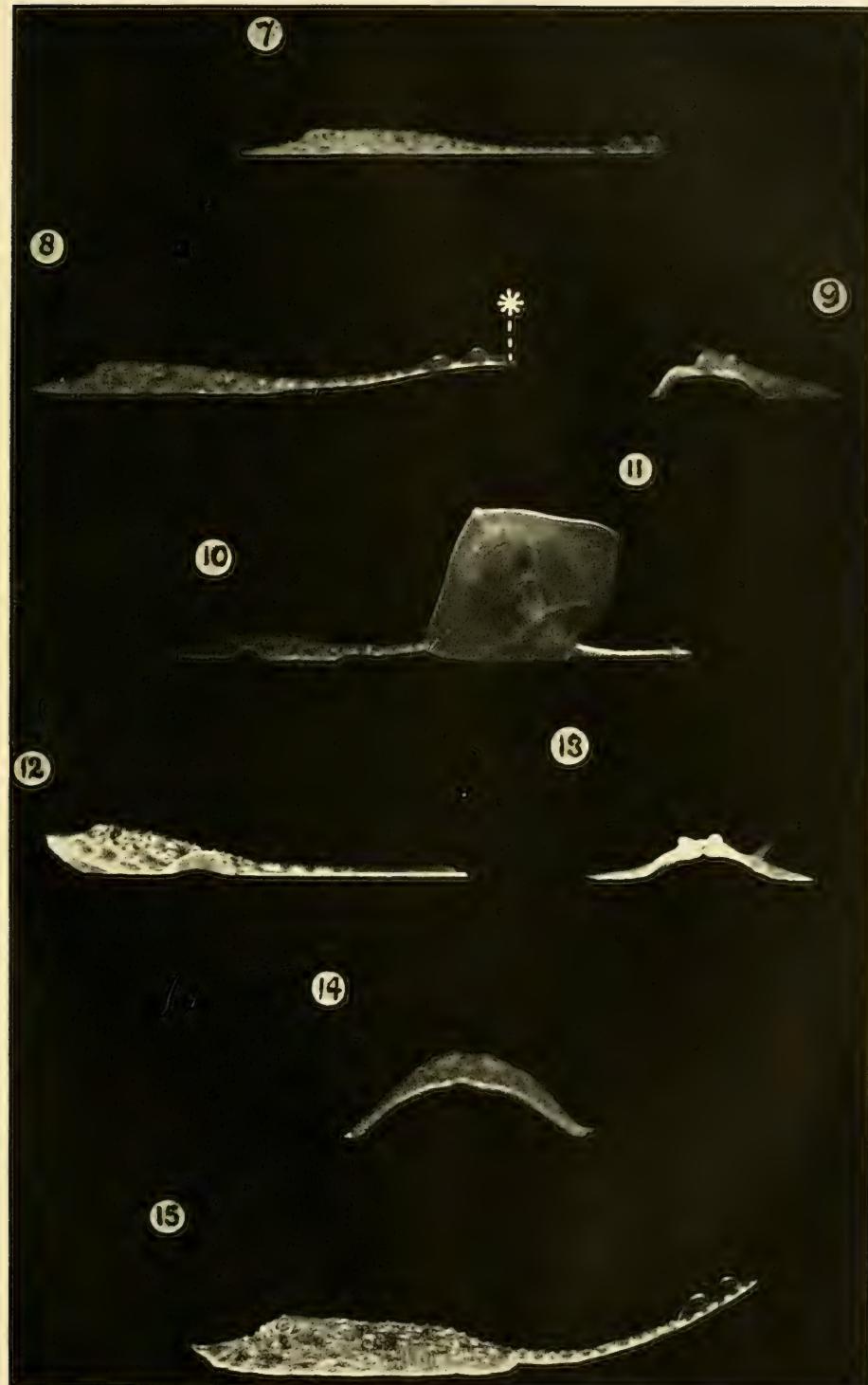
PLATE 3

16 Raia blanda (4). Part of a sagittal section through the filum terminale showing the free end of Reissner's fiber in front of the lesion (the posterior end to the left, in the figure). × 240.

17 Part of a transverse section through the spinal cord of the cat, showing a much swollen Reissner's fiber, nearly 10 μ in diameter, embedded in a mass of coagulum which almost blocks the central canal. In the center of the fiber can be seen the cut end of what appears as an axial thread or core. × 240.

18 Raia blanda (49). Part of a sagittal section through a piece of the spinal cord from the anterior part of the trunk, from which region Reissner's fiber had wholly retracted (caudally). The middle of the canal is occupied by a filmy structure (x) which is probably coagulum. × 240.

19 Raia clavata (XIX, not experimental). Part of a sagittal section through the end of the tail showing the broken end of Reissner's fiber in the (secondary) sinus terminalis. The terminal neural pore is almost choked by a mass of débris and coagulum. × 240.

20 Raia clavata (XXXIX, not experimental). The broken end of Reissner's fiber is seen loosely coiled near the end of the central canal, the anterior piece of fiber depicted having been added from an adjacent section. The (primary) sinus terminalis is fairly typical and extends downwards, in normal fashion, behind the end of the notochord. × 240.

21 Raia blanda (11). A length of Reissner's fiber from the fourth ventricle, to show the brittle condition of the preserved fiber, which has splintered upon the microtome knife. × 240.

22 Raia blanda (49). Part of a sagittal section through the filum terminale, immediately in front of the lesion. Reissner's fiber is seen entangled in a clot from which there has been no apparent retraction forward. Nevertheless, the whole length of fiber in the piece examined is spirally coiled, this being the result of a backward recoil from some point in the spinal cord. × 45.

23 Raia clavata (37). Part of a sagittal section through the filum terminale, in front of the lesion. Reissner's fiber is seen swollen and irregularly coiled. × 240.

ABBREVIATIONS

c.c., central canal of the spinal cord (and terminal filament)

cg., coagulum, practically filling the central canal (fig. 17)

cl., blood clot, in the central canal

e., epithelium lining the central canal

f.t., filum terminale

nch., notochord

R.f., Reissner's fiber

s.t., sinus terminalis

t.n.p., terminal neural pore

v.c., vertebral column

**, indicate the region of the incision
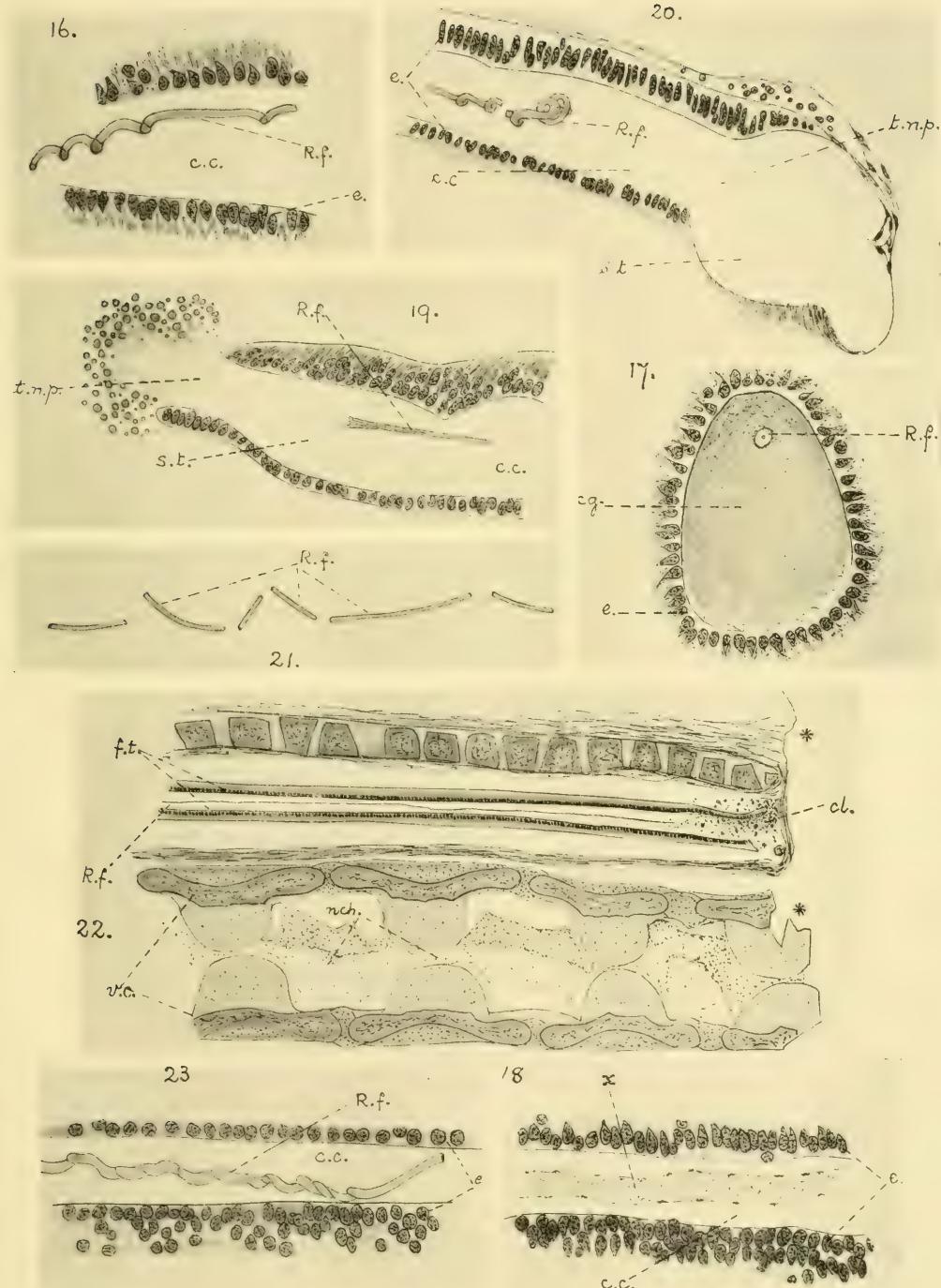
PLATE 4

24 Raia clavata (5). A transverse section (slightly obliquely cut) through the filum terminale, at a point a little in front of the sinus terminalis. Several fibrillae are seen which have apparently broken free from the displaced and slack Reissner's fiber. × 320.

25 Raia clavata (XIX, not experimental). Part of a sagittal section through the filum terminale. Posteriorly the fiber is seen swollen but fairly regular. It passes into a tightly tangled knot, from the anterior end of which it emerges, loosely coiled. (Posterior end to the left, in the figure.) × 240.

26 Raia blanda (10). Part of a sagittal section of the hinder end of the spinal cord, some half inch in front of the lesion. The posterior end of an extensive but loosely tangled skein of Reissner's fiber (of nearly normal diameter) is seen in the central canal which is cut obliquely. (Posterior end to the left in the figure.) × 320.

27 Raia clavata (64). Part of a sagittal section through the filum terminale in front of the lesion. Reissner's fiber is very fine and, to the left (anterior) of the figure is seen in apparent contact with the wall of the central canal. Behind this point it lies slackly, well away from the wall of the canal. × 320.

28 Raia clavata (18). Part of a sagittal section through the filum terminale, immediately in front of the lesion. Retraction of the fiber was prevented by the formation of an extensive clot (lying more anteriorly and not shown in the figure). The severed end of the fiber is seen fibrillated and flaring as though to produce a new terminal plug. × 240.

29. Salamandra maculosa. Part of a transverse section through the spinal cord. Reissner's fiber apparently receiving three or four constituent fibrillae. Near the dorsal line there projects a conical process which is probably the apex of a sensory cell. × 340.

30 Scyllium canicula (44). Part of a sagittal section through the filum terminale showing the unretracted fiber, with what are apparently constituent fibrillae in situ. × 340.

31 Scyllium canicula (2). Part of a sagittal section through the filum terminale at the point where this was severed by the experimental incision. The cut end has become rounded off and the meninges have grown around it to completely enclose the secondary sinus terminalis. The end of Reissner's fiber is seen flaring somewhat and has, apparently, made good its new attachment. × 340.

c.c., central canal of the spinal cord (and terminal filament)

d.b.v., dorsal blood vessel

e., epithelium lining the central canal

fb., fibrillae of Reissner's fiber in the central canal

f.t., filum terminale

mn., meninges, forming the hinder wall of the sinus terminalis

R.f., Reissner's fiber

s.p., sensory process (?)

s.s.t., secondary sinus terminalis

**, indicate the region of the incision
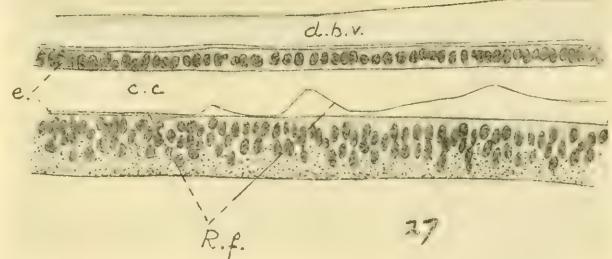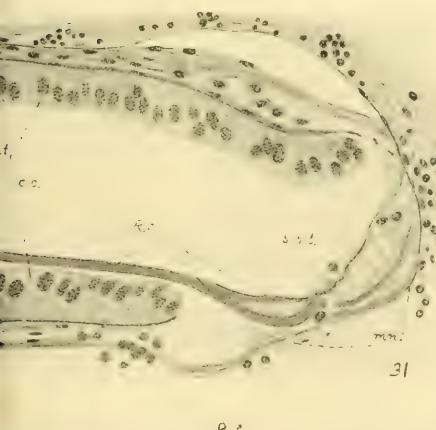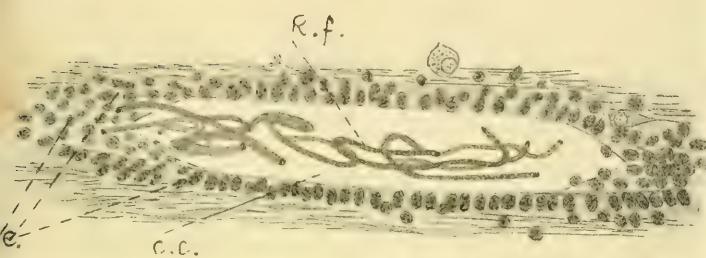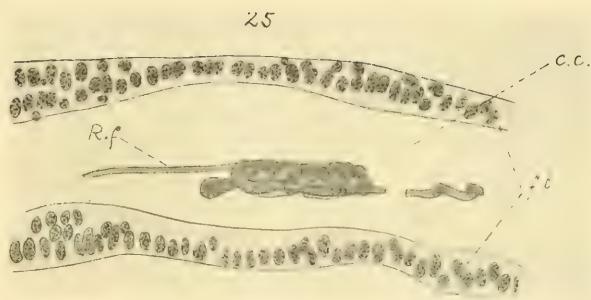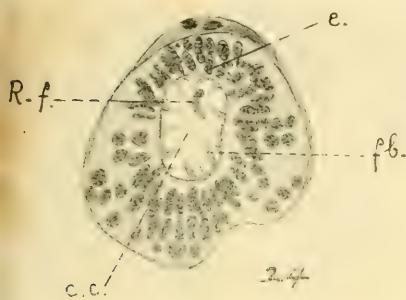
STUDIES ON THE OLFACTORY BULBS OF THE ALBINO RAT—IN TWO PARTS

I. EFFECT OF A DEFECTIVE DIET AND OF EXERCISE

II. NUMBER OF CELLS IN BULB

CAROLINE M. HOLT

From The Wistar Institute of Anatomy and Biology[1]

FOUR PLATES

PART I.   EXPERIMENTS TO DETERMINE THE EFFECT OF A DEFECTIVE DIET AND OF EXERCISE UPON THE WEIGHT OF THE OLFACTORY BULBS

CONTENTS

I. INTRODUCTION

The various members of the mammalian series show considerable variation in the relative development of all parts of the central nervous system, but probably no part of the encephalon shows so great a degree of variability as does the rhinencephalon. Of this portion of the brain, the olfactory bulbs are, without doubt, the most variable in size. Thus we have the very large bulbs of the opossum and the ant-eater; the almost rudimentary bulbs of the ape and of man; extreme reduction of these organs in the Cetacea, with their complete disappearance in the dolphin. Not only do we find variation in size of the olfactory bulbs among the different orders of mammals, but we find that there is a considerable degree of variability within each order and even among the members of the same species.

This variation in size and weight of the olfactory bulbs within a species is well illustrated by observations upon the rats in the colony of The Wistar Institute. The domesticated albino rats exhibit a considerable range in the development of this part of the brain. But while we find an appreciable difference in the bulb size of rats of different litters even under like environmental conditions, the individuals of a given litter usually show a more uniform development of the olfactory system. Some wild Norway rats examined at The Wistar Institute a few years ago had olfactory bulbs heavier in proportion to total brain weight than the bulbs of the albino. In the course of the present study, observations made upon some thirty wild Norway rats caught at different places in Philadelphia suggested that this difference between the two strains is not a constant one, for,

while the olfactory bulbs of these animals were much heavier than those of the albinos, the ratio between bulb weight and brain weight in this series was about the same in the two forms.

Inequality in the size of the two bulbs in the same individual appears not infrequently in the albino and when it occurs it will often be found in several, and occasionally in all, members of the same litter. For this reason, in selecting material for these experiments, we discarded all litters in which cases of asymmetry were observed among the initial controls.

Observations made from time to time by Dr. Donaldson, indicated that rats born in the early summer differ from winter-born rats in the relative size of the olfactory bulbs; also that there might be a difference between rats reared on a restricted diet, such as is frequently used in colonies, and those fed on the table-scrap diet adopted for the Wistar colony. Moreover, cases had appeared in which the bulbs of sick rats were apparently smaller than those of healthy individuals.

All these facts suggested that there might be factors in the living conditions of the rats which would account for the variability of this portion of the nervous system, the growth of the bulbs being retarded or arrested in rats reared under unfavorable conditions, such as the intense heat of the summer, or a monotonous diet, or in those suffering from the various infections which may attack the rats from time to time.

It was, therefore, with the hope of throwing some light upon the question of the effect of environmental conditions upon the olfactory bulb of the growing albino rat, that, at the suggestion of Dr. Donaldson, the present experiments were undertaken. The problem resolved itself into two questions—Can the growth of the olfactory bulbs of the stock albino be modified (1) by underfeeding or (2) by exercise?

II. DEFECTIVE DIET EXPERIMENTS

1. *Previous experiments on the effect of starvation upon the central nervous system of the rat*

There have been several previous studies upon the effect of underfeeding and of starvation upon the central nervous system of the albino rat. In 1904, Hatai reported experiments on 'partial starvation' for twenty-one days. He fed large quantities of starch with some fat but no proteids of any kind. He obtained a deficiency in body weight of 27 per cent in females and 32 per cent in males. Taking the values from the initial controls for a standard, the brains of the test rats showed, at the end of twenty-one days, a deficiency of 2.8 per cent for the females and 5.8 per cent for the males. Thus the treatment produced not only an arrest of brain growth but a loss in absolute brain weight. This experiment was followed by a series in which the animals, after a defective diet (Oswego starch), were returned to a normal diet. Here Hatai ('07) found that the effect of twenty-one days of partial starvation was eventually compensated for, so far as brain weight was concerned, but the central nervous system had suffered some change in its chemical composition. The following year ('08), Hatai published the results of further experiments, this time in quantitative underfeeding with an adequate ration, in which he concludes that growth in the stunted rats is just as normal as in the controls; i.e., all parts are proportionately stunted.

In 1911, Donaldson published an account of the effect of underfeeding, with a quantitatively deficient, but adequate ration, on the percentage of water, on the ether-alcohol extractives and on medullation in the central nervous system of albino rats, showing a slight diminution of percentage of water, slight increase in percentage of ether-alcohol extractives, and no notable difference in medullation.

Jackson ('15) found, in young rats maintained at a constant weight on a diet of bread and milk, that the relation between body weight and brain weight remained unchanged. The brain

ceased to grow simultaneously with the body, while the cord increased somewhat in weight during underfeeding.

Although there are, at present, no data by which it is possible to make a definite comparison between the effect of a defective diet and that of an adequate, but quantitatively insufficient diet, it is important to bear in mind in each case the method by which growth has been retarded or arrested.

Two series of experiments upon the effect of underfeeding were undertaken—one upon rats which had been reared to the time of weaning (at three or at four weeks of age) by well-fed mothers, Series A; the other, upon rats reared to the time of weaning by underfed mothers, which meant rats underfed practically from birth, Series B.

Observations were also made upon a few sick animals, Series C.

2. Series A. A_1, rats underfed from time of weaning at eighteen to twenty days, and A_2, at thirty to thirty-two days

a. Method. As has been previously stated, while there is a considerable range of variation between litters in the matter of the relative size of the olfactory bulbs, yet within a given litter the size is fairly uniform. For this reason, so far as possible, control and test animals were taken from the same litter. This, of course, made it necessary to select fairly large litters in order to have several animals for initial and final controls, and also for experiment. The litters were always taken from healthy stock animals.

For the first few individuals experimented upon, no initial controls were examined, but the results of these experiments made the advisability of such controls apparent and subsequently each litter was weighed and divided into three groups; so far as possible, equivalent in sex, weight, and bodily condition. All these rats were ear marked and a card filed for the data upon each animal.

The first or initial control animals were at once etherized, weighed, measured, eviscerated, and the brains removed. One olfactory bulb was cut off from each brain, in the following man-

ner. The brain was placed, ventral side down, on the dissecting board. Then with a thin, sharp scalpel held in a position perpendicular to the plane of the board and at right angles to the plane of the median longitudinal fissure, the bulb was severed just below the anterior limit of the cerebrum.[2] The bulb, with the remainder of the brain was then placed in a covered weighing bottle and the weight of both the entire brain and of the severed bulb ascertained.

The final controls were weighed and placed under the normal living conditions of the colony: i.e., housed, in long wooden cages with wire fronts, thick shaving-covered floors, and paper nests, and given plenty of fresh water with a carefully supervised scrap diet. The test rats were weighed and placed in adjoining cages under exactly the same conditions as the final controls, save for the diet. The food given the test rats consisted of an unlimited amount of whole corn, usually fed on the cob, save in case of very young animals, or those weak from a long period of underfeeding. In such cases, the corn was shelled as the animals were not able to remove a sufficient amount for themselves.

Both control and test animals were weighed from time to time and the weights recorded. Note was also made of any irregularities, such as a temporary change in diet, etc.

At maturity, a certain number of test and of control animals were mated in order to find out whether underfeeding affected the fertility of albino rats.

In the case of relatively small litters in which the members were usually well grown and in good physical condition when weaned—and especially if weaning was delayed until the rats were four weeks old—it was possible to keep the test animal on a corn diet for a month or more with practically no difficulty.

[2] Small bulbs tend to differ characteristically in shape from large ones. On section it is seen that the cap of gray substance extends somewhat further caudad on the ventral surface of the small bulb than it does in the case of the large bulb. The weight of gray substance thus lost in the case of the small bulb is a very small fraction of the total weight of the bulb but a much larger fraction of the gray cap. Care must, therefore, be taken to include this portion when the number of cells of the gray substance is to be determined.

With the rats weaned at three weeks or in case of small rats from very large litters, there was a good deal of trouble in keeping the animals on the corn diet for so long a time, and of course the difficulty increased as the period of underfeeding was prolonged.

At first an attempt was made to keep animals from several litters in one cage with the result that after a short time, the less well grown rats were killed and eaten by the stronger individuals. Then the plan was adopted of having members of only one litter in a cage. This worked successfully up to the time when the animals began to weaken. Then the males frequently killed and ate the females. So finally, for prolonged experiments, it was found safer to place only animals of the same sex, approximate weight and physical condition together, but even this precaution was not always sufficient.

In most cases, for the first few weeks, there was a very slow gain in weight or the weight was just maintained. But in every case when an animal began to lose or became very feeble, a dose of condensed milk was fed. One or two doses were usually sufficient to restore the animal to equilibrium and there was not infrequently a sudden temporary gain in weight, doubtless due to increased appetite and the consequent gorging of the alimentary tract with corn.

In a few cases where the underfeeding had gone on for several months, it became necessary to administer small doses of condensed milk more frequently—in two cases, practically every day—in order to keep the animals from losing weight.

At the end of the experiment, both test and final control animals were killed, weighed, measured, eviscerated, and brains and bulbs weighed as in the case of the initial controls. One bulb with a part of the cerebrum was preserved for histological study. A record was kept of any signs of disease or other abnormality. The weighing was done in closed bottles and all weights of brain and of olfactory bulbs were made to 0.1 mgm., but recorded here in milligrams only.

b. Results. General morphological and physiological modifications. A summary of the data from observations upon 108

individuals of Series A is given in tables 1 to 8. The complete tables with the records for each individual rat of this, as well as of the other series, are deposited at The Wistar Institute. Of the two litters weaned at eighteen and twenty days, only three individuals survived to be killed; the others died in the cages and the brains were not weighed. The records for the three rats just named have been included in tables 3 and 7, and their controls, with the corresponding controls. The size and body weight of rats weaned at the end of the third week and placed on a corn diet indicated clearly that under like conditions, rats weaned at three weeks are considerably more sensitive to adverse conditions than are those weaned at four weeks.

For every individual of Series A_1 and A_2 (tables 1 to 8), the stunting effect of the corn diet was apparent almost from the first. During the early weeks of underfeeding the test rats appeared rather more lively than the controls. Later this activity decreased, the gait became unsteady, and the animals appeared stupid. They were often unable to find the dish of condensed milk by themselves, whereas control rats would go to it immediately. This suggests that the underfed animals lacked an acute sense of smell and perhaps did not see clearly.

In every one of the test animals of which there are complete records, the general bodily growth was arrested by a diet of corn. This agrees with the observations of Osborne and Mendel ('13). These rats remained like young animals in appearance as well as in size. The earlier weaning took place and the corn diet was begun, the more complete the stunting.

The skeleton became modified and somewhat distorted owing to imperfect calcification. The growth of the long bones was not quite so completely arrested as that of the rest of the skeleton. The skull, sternum, and sometimes the ribs, became like parchment. In two cases the pressure of the heart upon the sternum had formed a sort of pocket out of that structure, which appeared like a tumor on the ventral side of the rat. The vertebral column became somewhat bowed, giving to the rat a 'humped' appearance and making it necessary to stretch the animals when measuring body length. One to four months of

underfeeding, following the first month under normal conditions, left the rats but slightly longer (4 to 10 mm.) than the initial controls measured at thirty days. The average increase in weight was in about the same proportion. Compare tables 2 to 8, for body weight and body length.

All the rats showed extreme emaciation but this condition was largely masked by the condition of the coats. The hair remained short and soft, with a fluffiness which gave even to mature rats the appearance of plump young animals. Such emaciation was, of course, accompanied by great muscular weakness. Rats kept for long periods on the defective diet became unable to remove corn from the cobs. They walked with a tottering gait and moved about but little.

The cyanosed condition of these animals was clearly indicated by the blue color of all exposed parts of the body—nose, ears, feet and tail. In protracted cases of underfeeding, a chronic palpitation of the heart developed which increased in violence as time went on. As a result of this, the whole body shook constantly.

All animals kept on corn up to maturity failed to breed or to show any sexual instinct whatever.

Effect on brain and olfactory bulbs (compare tables 1 to 8). In Series A, both A_1 and A_2 show a slight increase in brain weight during the period of underfeeding. Under normal conditions, as the rat grows, the brain becomes relatively lighter in proportion to body weight. In the underfed rats the brain forms practically the same proportion of the total body weight as in the initial control rats (agreeing with Jackson's results ('15)), which of course indicates in the cases where growth has taken place that the brain has not been as much arrested in its development as has the rest of the body.

After four to eight weeks of underfeeding, the rats of Series A_1 and A_2 had olfactory bulbs which, taken together, formed about the same proportion of the total brain weights as did the bulbs of the initial controls of the same series, showing that the relation of these parts of the brains had not been changed during the experiment. But normally the olfactory bulbs grow faster

during this period than the rest of the brain so that at eight weeks, for example, the bulbs should form a considerably greater percentage of the total brain weight than at thirty days. The average absolute weight of the bulbs of the test animals was equal to but 70 to 81 per cent of the average weight of the bulbs in the control animals of the same series. It is therefore evident that the retarding effect of underfeeding has been greater upon the olfactory bulbs than upon the other parts of the brain, which had 85 to 90 per cent of the weight of the brains in the control series.

If the relative weight of the bulbs in Series A_1 and A_2 is determined for the test group as contrasted with the final control group, we obtain the following relations:

TABLE 1

	GROUP	AGE	PERCENTAGE WEIGHT OF OLFACTORY BULBS
		days	
Table 3..........	Test rats, defective diet	60	3.52
Table 4..........	Final controls	60	3.99
Table 5..........	Test rats, defective diet	79	3.39
Table 6..........	Final controls	79	4.16
Table 7..........	Test rats, defective diet	118	3.83
Table 8..........	Final controls	128	4.30

This arrangement of the results shows clearly that in each of the three sets, grouped according to age, the olfactory bulbs of the underfed rats are significantly lighter in proportional weight than those of the controls. We may, therefore, conclude that the relative weight of the olfactory bulbs is reduced by the form of defective feeding employed in this experiment.

The details are given in tables 2 to 8, which follow.

3. Series B.   Rats on deficient diet from birth

a. Method.   Since it was evident that the earlier the animals were weaned, the greater the stunting effect of a qualitatively inadequate diet, it occurred to the writer that it would be interesting to try underfeeding from birth, by underfeeding the

TABLE 2. SERIES A

Initial control animals

In all of the tables the averages are weighted for the number of animals in each entry

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
14 males..................	30	44.1	118	1.439	0.051	3.52	3.01–3.97
11 females................	30	43.5	116	1.409	0.050	3.53	2.41–4.19
Averages for males and females..................		43.8	117	1.426	0.050	3.53	2.41–4.19

TABLE 3. SERIES A

Test animals

Stock albinos kept on corn diet for twenty-nine to forty-two days after weaning at three to four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
15 males..................	60	55.5	126	1.505	0.052	3.45	2.38–4.53
11 females................	60	53.9	126	1.502	0.054	3.62	2.68–4.21
Averages for males and females..................		54.8	°126	1.504	0.053	3.52	2.38–4.53

TABLE 4. SERIES A

Final control animals

Stock albinos kept on normal diet for twenty-nine to forty-two days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
19 males..................	61	127.4	167	1.668	0.066	3.95	2.76–4.62
7 females................	60	95.1	154	1.606	0.065	4.04	3.66–4.53
Averages for males and females..................		118.8	164	1.651	0.066	3.99	2.76–4.62
Summary $\frac{\text{Test}}{\text{Control}}$			77	91	81		

211

TABLE 5. SERIES A

Test animals

Stock albinos kept on corn diet for forty-nine days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
2 males....................	80	47.8	126	1.502	0.055	3.63	3.31–3.93
1 female....................	78	37.8	116	1.458	0.042	2.89	
Averages for males and females..................			124	1.487	0.050	3.39	2.89–3.63

TABLE 6. SERIES A

Final control animals

Stock albinos kept on normal diet for forty-nine days after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
2 males....................	80	155.0	178	1.727	0.068	3.93	3.72–4.14
1 female....................	78	151.2	183	1.703	0.079	4.63	
Averages for males and females..................		153.8	180	1.719	0.072	4.16	3.72–4.63
Summary $\frac{\text{Test}}{\text{Control}}$.........			69%	78%	70%		

TABLE 7. SERIES A

Test animals

Stock albinos kept on corn diet for fifty-nine days or more, after weaning at four weeks. (One rat weaned at eighteen days)

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
7 males....................	120	47.1	118	1.463	0.057	3.86	3:47–4.19
6 females..................	115	54.3	126	1.594	0.060	3.79	3.55–4.29
Averages for males and females..................		50.5	122	1.524	0.058	3.83	3.47–4.29

TABLE 8. SERIES A

Final control animals

Stock albinos kept on normal diet for fifty-nine days or more, after weaning at four weeks

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS	BULBS: PER CENT OF BRAIN WEIGHT	RANGE
	days	gm.	mm.	gm.	gm.		
8 males....................	121	201.4	199	1.806	0.078	4.29	3.81–4.73
4 females..................	136[1]	157.0	181	1.706	0.074	4.32	4.03–4.62
Averages for males and females.................		186.7	193	1.772	0.076	4.30	3.81–4.73
Summary $\dfrac{\text{Test}}{\text{Control}}$........			63%	85%	77%		

[1] This higher average age for the female controls is due to the fact that one female was kept for breeding purposes until two hundred and thirteen days old. As all her measurements were practically identical with those of another female of same litter, one hundred and fifteen days old, the record was included in the table.

mothers which were bearing or nursing the young to be tested. Consequently nine pregnant females were selected. A few were put on a corn diet several days before the birth of the young, but most of them began the corn feeding on the day of the birth of the litter. The young rats were weaned at three weeks and fed exclusively on corn.

It is intended to carry out this experiment more extensively at some future time but enough animals were tested to give significant results. It was found very difficult to raise such litters, for two reasons. In the first place, after the young reached an age to leave the nest, the mother was very apt to kill the entire litter. This, apparently, was not because of hunger, for in all but two cases in which the young rats were partially eaten, the animals were mutilated only to the extent of a bite through the cerebellum, and sometimes through the front of the throat. It has been suggested that the increasing demands of the young, coupled with an inadequate milk supply, may have been the cause of this unnatural behavior of the mothers.

But the chief reason for the difficulty in raising these rats was their lack of vitality. Although very active and playful, these animals were extremely frail little creatures, so weak that the slightest disturbance was likely to prove fatal. For example, an unusually active and promising test rat of fifty-three days, was carried from the colony to the laboratory for examination. As he appeared much excited, the carrier cage was set aside for an hour. The rat was heard running about for a time but at the end of the hour was found dead. The body weight of this rat was that of an animal fifteen days old and the brain weight was scarcely more. Young rats might appear lively and in every way normal in a late afternoon and be found dead in the cage next morning, for no reason to be discovered even after careful examination. Of nine such litters only two survived to the time of weaning, and these were kept with much difficulty.

A litter of 'runts' was also included in this series. This was a litter of rats, all of which failed to grow normally, presumably because the mother had an insufficient supply of milk. They appeared in every way like the rats which had been stunted by underfeeding the mothers.

b. Results. The general results of underfeeding in Series B were essentially the same in character as in Series A but they were considerably more marked (tables 9, 10 and 11). The body length and general appearance of seventy-seven day rats, underfed from birth, were practically the same as in normal three-weeks-old rats, save for the extreme cyanosed condition.

From a comparison of Series A_1, A_2, and B, it becomes evident that it is easier to retard the growth of an eighteen day rat than of a rat thirty days old, and still easier to stop the growth of a rat at about the size of an eighteen day individual if the underfeeding is begun at birth. Moreover, it is obviously far more difficult to rear these animals underfed from birth than rats which have been allowed to get a good start of thirty days under favorable conditions and are therefore much more resistant to the deleterious effects of partial starvation.

Effect on brain and olfactory bulbs. Series B shows brains actually lighter in weight at twenty-four to fifty-three days of

age than normal brains of seventeen days (The Rat, table 74).
Rats seventy-seven days old had brains weighing practically
the same as those of normal female rats of forty-two days.

Bulbs of rats twenty-four to fifty-three days old, actually
weighed only 70 per cent as much as those of normal rats of
thirty days (table 2) and only 52 per cent as much as bulbs of
normal rats eight weeks old (table 4).

Rats eleven weeks old (table 11) gave bulbs of the same abso-
lute weight as those of control rats of thirty days (table 2).
In both cases the olfactory bulbs formed a smaller per cent of
the total brain weight than appeared among the controls of
like age in Series A, as the following arrangement of the data
shows:

TABLE 9

	GROUP	AGE	PERCENTAGE WEIGHT OF OLFACTORY BULBS
		days	
Table 10.........	Test rats, defective diet- Series B.	24–53	3.14
Table 2.........	Control.	30	3.53
Table 11.........	Test rats, defective diet- Series B	77	3.48
Table 4..........	Control	60	3.99
Table 6..........	Control	79	4.16

The details for these series are given in tables 10 and 11 which follow.

4. Series C.   Sick rats

In the course of the experiments a number of sick rats came
under observation.   Eleven of these were examined to determine
whether the brain, and especially the olfactory bulbs, showed any
effects of the diseased condition.   Three of these rats were the
sole survivors from a group of twelve attacked by a serious
bowel trouble which killed the other nine occupants of the cages.
At the time of the onset of the illness, the rats were about eighty
days old.   After about ten days, these three seemed to recover
and were kept until they were about a hundred and thirty-five

TABLE 10. SERIES ,B

Test animals

Stock albinos underfed from birth.   Under two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
6 males............:...........	24–38	20.1	86	1.114	0.036	3.19	3.06–3.38
4 females.................	24–53	15.6	79	1.062	0.033	3.08	2.11–3.82
Averages for males and females.................		18.1	83	1.091	0.034	3.14	

TABLE 11. SERIES B 1.

Test animals

Stock albinos underfed from birth.   Over two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
3 females.................	77	31.2	102	1.437	0.050	3.48	3.15–3.93

days old when they were killed and examined. The other eight sick rats of this Series C were individuals showing a considerable infection of the lungs, and one of these (No. 20) had, in addition, a large abscess of the liver.

All of these rats were examined in the same way as those of Series A.

a. Results. In the group of sick animals, those with the intestinal infection had, at one hundred and thirty-four days, bulbs which averaged 0.050 gram or 3.02 per cent of the total brain weight (table 12, group 1) while a set of normal individuals of practically the same age gave an average of 0.073 gram or 4.32 per cent of the total brain weight (see table 20, group of females). These results seem especially interesting because here the adverse conditions appeared only after the rats were well grown—eighty days old—and lasted only about ten days.

The remaining two groups of sick rats all had infected lungs and were very old when killed. The two males had bulbs

averaging 0.037 gram, or 2.08 per cent of the total brain weight (table 12, group 2); while for the four females, the bulbs averaged 0.033 gram, 1.89 per cent of the total brain weight (table 12, group 3). For these last two groups there are no data of normal individuals for comparison but the percentage for the bulbs is strikingly low. Some unpublished data in Dr. Donaldson's hands show, however, that while the relative weight of the olfactory bulbs tends to increase up to about one hundred and fifty days of age, in older rats there is a tendency to decrease so that some of this decrease observed in the old sick rats (groups 2 and 3) may be due to normal age changes. But the remarkably small proportional weight of the bulbs here examined is probably due chiefly to the effect of disease.

In this connection may be mentioned two young rats of litter PR (group 2), killed at seventy days. Each had infected lungs. These rats came from parents with infected lungs and had lived since birth in a dark damp cage. One had very small unequal bulbs which were not weighed. The other had bulbs weighing only 0.019 gram or 1.30 per cent of the entire brain weight. This pair of bulbs were the smallest observed in the whole series of experiments. It seems quite evident that the bulbs are abnormal and quite probable that this abnormality is due to disease.

5. Summary and conclusions.   Defective diet experiments

1. General bodily growth in the albino rat is arrested by an exclusive ration of corn which constitutes a defective diet (Osborne and Mendel).

a. The skeleton is poorly calcified and somewhat distorted.

b. The muscular system is greatly reduced.

c. The coat has the appearance of that of a young animal.

2. Functional disturbances follow the arrested development.

a. There is increasing muscular weakness.

b. An increasing palpitation of the heart.

c. The animals appear cyanosed.

TABLE 12.   SERIES C

Sick animals

Females

Group 1.   Three albino rats from a lot of twelve controls for revolving cage experiment.   At about eighty days all contracted a severe bowel trouble from which these three recovered.

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT
	days	*gm.*	*mm.*	*gm.*	*gm.*	
Z$_1$.................	135	128.5	165	1.607	0.033	2.03
Y$_{22}$.................	135	150.5	180	1.534	0.034	2.24
X$_3$.................	134	157.9	182	1.803	0.082	4.55
Average females....	....	145.6	176	1.648	0.050	3.02

Males

Group 2.   Two, old, with infected lungs.

PR$_1$.................	70	88	148	1.489	0.019	1.30
PR$_6$.................	70	90	155	1.538	unequal	
No. 24.............	old	213	202	1.759	0.050	2.81
No. 69.............	365	214	195	1.791	0.024	1.35
Average Numbers 24 and 69........	....	213.5	198	1.775	0.037	2.08

Females

Group 3.   Infected lungs, old age, and in case of No. 20, bad abscesses on liver.

No. 20.............	340	143.0	173	1.673	0.027	1.63
No. 21.............	340	146.1	187	1.751	0.032	1.79
No. 71.............	370	186.0	186	1.687	0.051	3.01
No. 72.............	240	218.3	199	1.794	0.021	1.16
Average females....		173.4	186	1.726	0.033	1.89

d. The sense organs become dulled after prolonged defective feeding—the animals respond but slowly to stimulations of sound, or light or smell.

e. Defectively fed animals fail to breed.

3. The effect of defective feeding on the brain and olfactory bulbs is less than upon the rest of the body, but is, nevertheless, very marked.   The olfactory bulbs are stunted and to a con-

siderably greater degree than is the entire brain. When defective feeding is begun in rats about thirty days of age, the bulbs of rats thus experimentally stunted form about the same percentage of the total brain weight as do the bulbs of rats of the same litters killed at the beginning of the experiment. Whereas, under normal conditions, the bulbs of older rats (up to one hundred and fifty days) are considerably heavier in proportion than those of the young animals. With prolonged defective feeding the proportional weight of the bulbs tends to become slightly greater.

4. Sick animals, especially those with lung infection, show a marked diminution in the relative weight of the olfactory bulbs, accompanied by a certain amount of loss in total brain weight.

III. EXERCISE EXPERIMENTS

1. Previous experiments on the effect of exercise upon the albino rat

Several investigators have worked upon problems connected with the changes in the albino rat occasioned by an increased amount of exercise. J. R. Slonaker in 1907 published observations upon four rats of different ages kept in revolving cages for a short period. In 1912, the same author published an account of further experiments along the same line, and although this time, also, the work was with a small group of rats, yet the experiment was continued during the natural life of the animals. Slonaker was working chiefly upon the problem of normal activity in its relation to age and sex but, incidentally, he made some few observations upon the comparative development of 'exercised' and normal rats. He found that "exercised rats are more active, more alert, and brighter in appearance than the control ones," but that "the control males reach their maximum weight at an earlier age than exercised males, and also greatly excel them" and that "control rats live longer than exercised rats." No observations were made on the effect of exercise upon any of the internal organs.

Donaldson, in 1911, conducted a series of experiments to ascertain the effect of exercise upon the central nervous system

of the albino rat, using the same sort of apparatus—a revolving cage with cyclometer attachment—employed by Slonaker. He found that there was a slight increase in brain weight (2.4 to 2.7 per cent) to be attributed to the effect of exercise. This was what was to be expected in view of the heavier brain to be found in the wild Norway rat. The cord showed no effect. The olfactory bulbs were not weighed separately.

Hatai ('15) published a series of observations based upon his own experiments and upon those of the present writer, showing the rather marked effect of the same exercise conditions upon the weight of the internal organs. In these experiments, the brains of the test animals showed an excess of 4 per cent over the controls with no effect upon the cord.

2. Description of Experiments. Series D and E

As it had thus been demonstrated that the brain of the albino rat could be modified by exercise in the revolving cage, it remained to determine whether, under such conditions, the olfactory bulbs would show a more marked variation than the brain as a whole.

For this work, also, large litters of stock albinos, were chosen. Each litter was weaned and divided into three groups when about thirty-five days old. One group constituted the 'Initial Controls,' and these were killed and examined as in the previous experiments. The second lot, the 'Final Controls,' was set aside in cages under the normal living conditions of the colony. The third group was used for the experiment. Each of these test animals was placed by itself in a wire revolving cage such as had been used by Slonaker, and later by Donaldson and Hatai. Each cage was 5 feet in circumference with an open nest box fastened to the central fixed axis. From this axis the food was suspended so that, theoretically, the rat must descend to the floor of the cage to eat. Practically, some rats soon learned to avoid this and so escaped a considerable amount of enforced exercise.

Each cage was provided with a cyclometer. Readings were made and recorded six times a week. These cyclometer read-

ings showed only the activity of the rats when the cage revolved and were therefore incomplete, since some rats learned to play from side to side of the cage and keep it from revolving, while others learned to run up the middle of the sides in such a way as to hold the cage at rest. But most of the rats soon learned to run the cages and appeared to enjoy it.

The rats were fed on the same diet as the controls and all the animals were weighed at intervals of about two weeks.

3. Series D. Rats in revolving cages for thirty days

There were but two litters in this series. One litter was weaned and set aside at thirty-five days of age and the other at forty days. Both litters were subjected to exercise in the revolving cages for a period of only thirty days. All were killed at the end of the thirty days of exercise.

a. Results. The exercised males of these two litters gained more rapidly in both weight and body length than did the controls, while the females fell behind. The superior growth of the test males was sufficient to bring the averages for both males and females up to 113 per cent of the weight of the controls and to 104 per cent of the length (tables 13 and 14).

The records of the activity of Series D were accidentally destroyed, but as these were for a period of but thirty days, they would be of little value save in adding further evidence that the female rat becomes active sooner than the male.

While, on the average, there is no difference in the absolute brain weight of the test rats in Series D from that of the controls, when both are compared with the reference table values in The Rat (Donaldson, '15), according to the method there suggested (pp. 4 and 5), yet I believe the bulbs do show, even after this short period, some effect of the unusal activity (tables 13 and 14). In the females, the bulbs make up 4.46 per cent of the brain weight in test rats as compared with 4.36 per cent in the controls. With the males, the difference was more marked—4.55 per cent in tests to 4.20 per cent in controls, making a joint average for males and females of 4.51 per cent in tests against 4.32 per cent

TABLE 13. SERIES D

Test animals

Albino rats kept in revolving cages for thirty-three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	grams	mm.	grams	grams	
PR₁.................	70	88	148	1.489	0.019[1]	1.30
T₃.................	68	131.7	170	1.871	0.079	4.21
T₂.................	68	140.9	176	1.831	0.085	4.63
PR₃....'..........	70	173.2	185	1.784	0.085	4.79
Average males.......		148.6	177	1.829	0.083	4.55

Females

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
T₁.................	68	102.9	158	1.744	0.079	4.53
PR₂.................	70	116.0	162	1.653	0.073	4.39
Average females......		109.5	160	1.698	0.076	4.46
Average males and females...........		132.9	170	1.776	0.080	4.51

[1] Lungs infected. Rat undersized in every way, therefore not included in averages (Series C, Sick rats, p. 218).

in controls, the olfactory bulbs of the former being, therefore, 7 per cent heavier than those of the latter.

4. Series E. Rats in revolving cages for ninety-eight to one hundred and three days

The test animals of this group were kept in the revolving cages for fifteen weeks. At the end of that time, three pairs of test animals and one pair of controls were mated (brother to sister in each case). Some digestive trouble appeared in the cages of control rats rather early in the experiment and most of the rats died, while the remaining animals failed to attain a normal growth, so that satisfactory final controls were lacking for this group. But the rest of the test animals and the surviving controls were killed at the end of the fifteen weeks, measured,

TABLE 14. SERIES D

Final controls

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR$_5$..................	70	100.7	154	1.636	0.061	3.72
PR$_6$..................	70	90.0	155	1.538	Bulbs unequal[1]	
T$_4$..................	68	132.4	170	1.802	0.084	4.64
Average males.......		116.6	162	1.719	0.072	4.20

Females

T$_5$..................	68	110.8	159	1.780	0.081	4.52
T$_6$..................	68	115.6	165	1.774	0.077	4.32
PR$_7$..................	70	130.5	170	1.704	0.074	4.32
Average females......		119.0	165	1.753	0.077	4.39
Average males and females...........		118.0	164	1.739	0.075	4.32
$\frac{\text{Test}}{\text{Control}}$			103.7%	102.1%	106.8%	

[1] Lungs slightly infected. Not included in average.

weighed, examined, and bulbs preserved exactly as in the under-feeding experiments.

The mated animals were kept about one hundred days longer to see whether the exercise of the previous weeks would show any effect upon fertility.

a. Results. General body growth. We find by examination of the records of body weight taken at two-week intervals during the experiment, that up to the time the larger set of control rats fell sick, the exercised animals were gaining less rapidly in weight than were the controls. From the time of the illness, some five weeks after the beginning of the experiment, the control rats fell off in weight, and with a single exception, they never recovered. Litter W escaped the infection and the weight records for the six rats composing it are as follows:

TABLE 15

Record for Litter W, Series E, showing gain in weight for individuals in revolving cages and for controls

	TEST RATS			CONTROL RATS		
	W_6 (m)	W_4 (f)	W_1 (f)	W_2 (m)	W_3 (m)	W_8 (f)
Initial weight........	44.0 g.	40.5 g.	50.5 g.	43.5 g.	50.0 g.	40.5 g.
2 weeks weight......	60.4	62.0	65.6	63.2	68.7	69.2
4 weeks weight......	97.0	82.0	103.0	107.0	120.0	89.0
6 weeks weight.·.....	139.0	124.0	142.2	139.0	148.8	119.0
9 weeks weight......	186.0	142.0	170.5	194.0	212.0	148.0
30 weeks weight......	210.0	148.0	187.0	205.0	224.0	150.0
Final length.........	209 mm.	182 mm.	198 mm.	207 mm.	200 mm.	190 mm.

The test rats from Litter W were, on the whole, slightly longer and lighter in weight than the control animals. The majority of individuals in Litter W proved to have abnormal brains— one or both olfactory bulbs being very much undersized. The brains, therefore, could not be used for comparison and the litter was excluded from the tables. For comparison with the rest of the litters of Series E, it was necessary to use other stock litters, as will be described later (tables 18 to 25). The comparisons are not, therefore, of as much value as they would be were the controls from the same litter. On the average we find body length slightly more, and body weight slightly less, in test animals (table 25). I think we may conclude that these results agree in general with those of previous investigators indicating that exercise has but a slight effect, if any, upon either body weight or body length.

The size of the viscera was considerably modified. These results have been incorporated in the report by Hatai ('15).

Activity of exercised animals. These rats showed great individual difference in the amount of activity and in the age at which they became most active (tables 16, 19, 21, 25). In these respects, there was also a considerable difference in litters as shown by the following record.

If we take the record of these same rats for ninety-three days we get an average of 5.76 miles per day for males, and 5.96 miles

TABLE 16

Activity record of rats in revolving cages for one hundred and three days.   Series E

ANIMAL	TOTAL MILES	MILES PER DAY	ANIMAL	TOTAL MILES	MILES PER DAY
Y$_7$M............	914.5	8.9	Y$_1$M............	559.7	5.4
Y$_8$F............	770.5	7.5	Z$_3$M............	476.3	4.6
Y$_6$F............	724.0	7.0	X$_6$M............	470.8	4.6
Y$_4$F............	705.3	6.8	X$_2$F............	458.7	4.5
Y$_3$M............	689.0	6.7	Z$_6$F............	457.1	4.4
Z$_5$M............	577.8	5.6	Z$_4$F............	446.6	4.3
Average for males...	614.7	5.96			
Average for females	593.7	5.76			

for females, and if we go back still further we get a still higher average for the females and lower for the males.   The males were slow to begin to run the cages.   An extreme example, Y$_1$ of the present series, ran less than 2 miles during the first five weeks in the cage, but became extremely active during the last four or five weeks making a final average of 5.4 miles per day, a record almost equal to the average for the entire lot of males. The females soon learned to run the cages and became very active at an early age.   During the last weeks of the experiment, the activity of practically every female in the series was on the decline.   I think from a study of all the records it may be concluded that while, in the revolving-cages, the females reach the period of greatest activity earlier than do the males, yet in the long run, the records of a large number of males and females would average about the same.

Possible effect on fertility.   There is some indication that the fertility of the albino rat is increased by exercise.   In the cases of the three pairs of exercised rats which were mated, the following record of offspring was obtained, together with the record of one control pair.

The average size of litter for normal stock albinos has been found to be between 6 and 7 individuals (Donaldson '15).   This is about the average for the control pair, while the averages for the three test pairs is considerably higher—13, 10.5, and 9.

TABLE 17

TEST PAIRS	CONTROL PAIR'

W_1 21st day after mating, 12 young
and   61st day after mating, 11 young
W_6 102d day after mating, 16 young
         pregnant
	W_8 24 days after mating, 3 young
	and 50 days after mating, 12 young
	W_2 102 days after mating, 0 young
	         not pregnant

Y_6 22d day after mating, 9 young and 67th day after mating, 9 young Y_3 102d day after mating, 0 young not pregnant	
Z_4 22d day after mating, 12 young and 88th day after mating, 9 young Z_3 102d day after mating, 0 young not pregnant	

It is significant also that the pair making the record of an average of 13 per litter for three successive litters, and the control pair are from the same original litter. Of course the numbers here are too few to enable one to draw conclusions but it would not be surprising to find some correlation between the greater weight of the sex organs in the exercised rats (Hatai '15) and the fertility of these animals.

Effect on brain and olfactory bulbs. It has already been noted that most of the control rats of this series were lost through disease. For comparison with the exercised rats, a set of controls used in Series A of the defective feeding experiment was chosen (table 20). These rats seemed better suited for the purpose than any others because they had been born at the same season as the test animals and reared in the same laboratory, so the food from day to day was the same for the two sets of rats. Among these, it was possible to find records of eight rats of almost the same body length and weight and of approximately the same age as the exercised rats killed at the end of the experiment. For the four which were mated and not killed until they were two hundred and thirty-eight days old, it was not possible to get controls of the same age, the four oldest of the controls (table 22) averaging only one hundred and sixty-nine days and the body length being 6 per cent less than that of the test animals. But as these are beyond the one hundred and fifty day limit, up to which time the bulbs increase in rel-

ative weight, the difference is not so serious a matter as it would be were the rats younger.

The set of test animals killed at the end of one hundred and three days of exercise, gave bulbs averaging for the males 4.28 per cent of the entire brain weight, and 4.60 per cent for the females—an average of 4.41 per cent for the entire set (table 19). When these results are compared with the controls (table 20) we find that while the test animals were 1 per cent shorter than the controls and had brains 2 per cent lighter in weight, the olfactory bulbs were 3 per cent heavier. These results seem to indicate that the olfactory bulbs of the test animals have been affected by exercise.

An examination of the records for the initial controls of the litters concerned seems to give additional weight to this supposition. See table 23 below.

TABLE 18. SERIES E

Initial control animals

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	grams	mm.	grams	grams	
W₁..................	30	26	99	1.338	0.052	3.89
Y₁₀.................	30	36	104	1.254	0.052	4.18
W₇..................	30	41	109	1.472	0.056	3.83
Z₈..................	30	41	112	1.444	0.050	3.46
Z₇..................	30	46	121	1.452	0.056	3.87
Average males.......	...	38	109	1.392	0.053	3.84
Females						
Y₅........•.........	30	29	96	1.21	0.043	3.57
X₅..................	30	24	96	1.280	0.045	3.52
X₁..................	30	28	101	1.338	0.034	2.51
W₆..................	30	34	107	1.335	0.043	3.22
W₅..................	30	43	114	1.432	0.048	3.34
Average females......		31	103	1.319	0.043	3.22
Average males and females............		35	106	1.356	0.048	3.54

CAROLINE M. HOLT

TABLE 19. SERIES E

Test animals

Albino rats kept in revolving cages for one hundred and three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	OLFAC-TORY BULBS: PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
	days	*grams*	*mm.*	*grams*	*grams*		
X$_6$.................	134	233	193	1.927	0.079	4.12	4.6
Y$_1$.................	135	188	195	1.693	0.070	4.16	5.4
Z$_5$.................	135	226	198	1.849	0.080	4.30	5.6
Y$_7$.................	135	237	205	1.875	0.085	4.54	8.9
Average males.......		221	198	1.836	0.079	4.28	6.1

Females

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFAC-TORY BULBS WEIGHT	OLFAC-TORY BULBS: PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
X$_2$.................	134	151	177	1.661	unequal		4.5
Y$_3$.................	135	157	181	1.655	0.081	4.80	7.5
Z$_6$.................	135	162	180	1.796	0.078	4.32	4.4
Y$_4$.................	135	169	191	1.693	0.079	4.64	6.8
Averages females....		162	184	1.715	0.079	4.60	5.8
Average males and females............		196	192	1.784	0.078	4.41	5.95

As we see, the brains of the initial controls for the test animals (X, Y, Z) averaged but 91 per cent of the weight of the initial controls for the final controls (L, N, O, T, U, V); the olfactory bulbs but 89 per cent. Since it has been found that brain and olfactory bulb weight are pretty uniform for any given litter, and that when we find light or heavy brains or bulbs in the initial controls, we are fairly sure of finding the same relative development in the adult animals of the same litters, it seems fair to assume that normal adult individuals of litters X, Y, Z, would have had relatively lighter brains and bulbs than were found in adults of litters L, N, O, T, U, and V. If this assumed relation were true, then the results given in tables 19 and 20 doubtless would fall into line with those of previous experiments in which exercised rats showed an increase in brain weight over the

TABLE 20. SERIES E

Final control animals[1]

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
T₆.................	160	223	202	1.890	0.085	4.51
U₃.................	146	228	202	1.987	0.081	4.06
N₈.................	121	203	205	1.825	0.072	3.93
L₆.................	157	274	218	2.021	0.077	3.81
Average males.......		232	206	1.931	0.079	4.08

Females

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
H₈.................	93	127	172	1.559	0.063	4.03
V₇.................	124	144	177	1.672	0.067	4.03
T₇.................	213	183	187	1.801	0.082	4.56
O₁.................	115	175	188	1.792	0.083	4.62
Average females......		157	181	1.706	0.074	4.32
Average males and females...........		195	194	1.818	0.076	4.20
Series A $\frac{\text{Test}}{\text{Control}}$				99%	98%	103%

[1] Data from stock Albinos used for controls in Defective Feeding Series A and again used for comparison here, since the original controls died early in the experiment.

controls, and would indicate an even greater gain in bulb weight for the test animals than is indicated in the tables.

In the same way, we may compare the initial controls for the mated test animals and those for Series A used for a standard (tables 21 and 22). We find the initial relations practically the same as for the group just discusesd.

In the final results (tables 21 and 22) we see that although the test rats were older, with bodies 6 per cent longer, the brains were actually 5 per cent lighter in weight. Here again, examination of the initial controls suggests that in all probability there was not an actual loss of brain weight in the exercised animals.

TABLE 21. SERIES E

Test animals

Albino rats kept in revolving cage for one hundred and three days after weaning. At end of that time mated and allowed to rear 2–3 litters. Age, when killed, about eight months.

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
	days	grams	mm.	grams	grams		
Z₃.................	238	299	221	2.018	0.092	4.54	4.6
Y₃.................	238	311	228	1.842	0.094	5.10	6.7
Average males.......		305	225	1.930	0.093	4.81	5.7

Females

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT BRAIN WEIGHT	AVERAGE NUMBER MILES PER DAY
Z₄.................	238	216	202	1.777	0.080	4.58	4.3
Y₆.................	238	156	203	1.654	0.080	4.81	7.0
Average females.....		186	203	1.716	0.080	4.66	5.7
Average males and females...........		246	214	1.823	0.086	4.74	5.7

TABLE 22. SERIES E

Control animals

Stock albinos used for control in defective feeding experiment, Series A. The four oldest of this set chosen for present tests since original controls died early in the experiment.

AVERAGE	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS PER CENT OF BRAIN WEIGHT
U₃.................	146	228	202	1.987	0.081	4.06
L₆.................	157	274	218	2.021	0.077	3.81
T₆.................	160	223	202	1.890	0.085	4.51
T₇.................	213	183	187	1.801	0.082	4.56
Average..............		227	202	1.925	0.081	4.23
Series A $\frac{\text{Test}}{\text{Control}}$....			106%	95%	106%	

TABLE 23

INITIAL CONTROLS FOR TEST ANIMALS				INITIAL CONTROLS FOR CONTROL ANIMALS. (DEFECTIVE FEEDING EXPERIMENT)			
Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight	Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight
	grams	*grams*			*grams*	*grams*	
X, Y, Z............	1.305	0.047	3.60	L, N, O, T, U, V.	1.431	0.058	3.69
Test / Control	91%	89%					

TABLE 24

INITIAL CONTROLS FOR TEST ANIMALS				INITIAL CONTROLS FOR CONTROL ANIMALS (DEFECTIVE FEEDING EXPERIMENT)			
Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight	Litters	Average brain weight	Average olfactory bulbs weight	Per cent of brain weight
	grams	*grams*			*grams*	*grams*	
X and Y..........	1.340	0.051	3.76	L, U and T	1.458	0.055	3.76
Test / Control	92%	92%					

But, be this as it may, we find the bulbs of these test animals actually 6 per cent heavier than those of the controls, the bulbs making 4.74 per cent of the total brain weight, while those of Series A controls were only 4.23 per cent of the total weight of the brain.

Since we have no true control series for comparison, we can not, of course, draw conclusions as to the absolute gain in brain weight after exercise. But of the gain in olfactory bulb weight in exercised animals, there seems to be no doubt.

When we turn to table 25 and note that the average percentage weight for the bulbs in case of 29 normal rats (59 to 83 days old) is 4 per cent, while a study of table 13 shows there was no rat there recorded (save one sick one) in which the per cent fell below 4.21 per cent, while the average was 4.51 per cent, we must be convinced, I believe, of the reality of the effect of exercise. For the older rats, likewise, when we compare tables 19 and 25,

TABLE 25

(Summary) Showing comparative development of the olfactory bulb

Stunted series

TABLE	NO.	HISTORY	AGE	BODY WEIGHT	BODY LENGTH	AVERAGES FOR BRAIN WEIGHT	BULBS WEIGHT	BULBS PER CENT BRAIN WEIGHT	RATIO AVERAGE TEST TO AVERAGE CONTROL IN			AVERAGE MILES PER DAY
									Body length	Brain weight	Bulbs weight	
			days	*grams*	*mm.*	*grams*	*grams*		*per cent*	*per cent*	*per cent*	
II and XVIII....	38	Initial control. Underfed and Exercise Experiment	3; 18–20 35; 29–34	40	111	1.400	0.050	3.54				
X..........	9	Underfed from birth	27–53	18	83	1.091	0.034	3.14				
XI..........	3	Underfed from birth	77	31	102	1.437	0.050	3.48				
III..........	26	Underfed 29–32 days	59–62	57	126	1.504	0.053	3.52	77	91	81	
IV..........	26	Controls for above	59–62	119	164	1.651	0.066	3.99				
V..........	3	Underfed 49 days	78–83	44	124	1.487	0.050	3.39	69	87	70	
VI..........	3	Controls for above	78–83	154	180	1.719	0.072	4.16				
VII..........	13	Underfed 59–130 days	93–160	51	122	1.524	0.058	3.83	63	85	77	
VIII..........	12	Controls for above	93–160	187	193	1.772	0.076	4.30				

Sick animals

TABLE	NO.	HISTORY	AGE	BODY WEIGHT	BODY LENGTH	AVERAGES FOR BRAIN WEIGHT	BULBS WEIGHT	BULBS PER CENT BRAIN WEIGHT	Body length	Brain weight	Bulbs weight	AVERAGE MILES PER DAY
XII..........	3	Bowel infection	134–135	146	176	1.647	0.050	3.02				
XII..........	8	Infected lungs	70–370	187	190	1.742	0.034	1.96				

Exercise series

TABLE	NO.	HISTORY	AGE	BODY WEIGHT	BODY LENGTH	AVERAGES FOR BRAIN WEIGHT	BULBS WEIGHT	BULBS PER CENT BRAIN WEIGHT	Body length	Brain weight	Bulbs weight	AVERAGE MILES PER DAY
XIII..........	6	Revolving cage 30 days	68–70	133	170	1.776	0.080	4.51	103.7	102.1	106.8	
XIV..........	6	Controls for above	68–70	118	164	1.739	0.075	4.32				
XIX..........	8	Revolving cage 103 days	134–135	196	192	1.784	0.079	4.41	99	98	103	5.95
XX..........	8	Controls for above	93–213 (av. 141)	195	194	1.818	0.076	4.20				
XXI..........	4	Revolving cage 103 days and mating	237	202	214	1.823	0.086	4.74	106	95	106	5.65
XXII..........	4	Controls	146–213 (av. 169)	227	202	1.925	0.081	4.23				

we see that the average for 12 controls (90 to 160 days old) was 4.26 per cent while only two test animals fell as low as this (one of these was of abnormally light body and brain), and the averages were 4.41 per cent and 4.74 per cent for four and one-half months and eight months respectively.

5. Summary

1. The results of the present experiments agree with those of previous investigators in that they show no marked effect of exercise either upon body length or body weight in the albino rat.

2. The female albino becomes very active earlier than does the male but the activity of the male later increases to such an extent that the total activity for the two sexes for long periods is probably about equal.

3. These experiments suggest that there is an increase in fertility correlated with increase in the size of the reproductive organs.

4. The brain weight is slightly increased by exercise.

5. The weight of the olfactory bulbs of albino rats exercised in revolving-cages for periods of from thirty to one hundred days, is considerably increased. The bulbs of such rats form from 4.41 to 4.74 per cent of the total brain weight as compared with 4.20 to 4.32 per cent in rats reared under normal colony conditions. These bulbs show an increase of 5 to 11 per cent over and above the increase in weight manifested by the entire brain.

IV. CONCLUSIONS

From the preceding observations we may conclude that we are able to modify the olfactory bulbs of the rat by changing the conditions under which it lives and to modify them to a considerably greater degree than we can change the rest of the brain. In cases of stunting, the bulbs tend to overcome the effect, to a certain extent, as time goes on. With exercise the effect seems to increase with age. Yet the bulbs respond more markedly to the stunting effect of defective feeding or sickness than to the stimulating effect of exercise.

A histological study of these modified bulbs will be presented in the second part of this paper.

V. LITERATURE CITED

DONALDSON, H. H. 1911 On the influence of exercise on the weight of the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, pp 129–137.

1911 a The effect of underfeeding on the percentage of water, on the ether-alcohol extractives, and on medullation in the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, pp. 139–145.

1915 The Rat. Memoirs of the Wistar Institute of Anatomy and Biology, No. 6.

HATAI, S. 1904 The effect of partial starvation on the brain of the white rat. Am. Jour. Physiol., vol. 12, pp. 116–127.

1907 Effect of partial starvation followed by a return to normal diet, on the growth of the body and central nervous system of albino rats. Am. Jour. Physiol., vol. 18, pp. 309–320.

1908 Preliminary note on the size and condition of the central nervous system in albino rats experimentally stunted. Jour. Comp. Neur., vol. 18, pp. 151–155.

1915 On the influence of exercise on the growth of organs in the albino rat. Anat. Rec., vol. 9, no. 8, pp. 647–665.

JACKSON, C. M. 1915 Changes in young albino rats held at constant body weight by underfeeding for various periods. Anat. Rec., vol. 9, pp. 91–92.

1915 a Changes in the relative weights of the various parts, systems, and organs of young albino rats held at constant body weight by underfeeding for various periods. Jour. Exp. Zool., vol. 19, pp. 99–156.

OSBORNE, T. B. AND MENDEL, L. B. 1911 Feeding experiments with isolated food substances, Part 2.

SLONAKER, J. R. 1907 The normal activity of the white rat at different ages. Jour. Comp. Neur., vol. 17, pp. 342–359.

1912 The normal activity of the albino rat from birth to natural death, the rate of growth, and the duration of life. Jour. Animal Behavior, vol. 2, pp. 20–42.

PART II. ON THE NUMBER OF NERVE CELLS IN LARGE AND SMALL OLFACTORY BULBS

CONTENTS

I. INTRODUCTION

The foregoing studies have shown that it is possible to change the relative weight of the olfactory bulbs in the albino rat (Holt, '17). This relative weight is decreased by a defective diet and increased by exercise. Such being the case, it seemed very desirable to make a histological comparison of the bulbs which had been stunted by a defective diet or enlarged by exercise, with those of rats reared under normal colony conditions. For this purpose it was of course essential to find a method of fixation and treatment which would give uniform results. Fixation in Ohlmacher's solution as recommended by King ('10) for the study of cortex cells, gave very satisfactory results, but her statement that "various individuals react differently although subjected to the same course of treatment," and her tables (loc. cit., p. 231) showing a variation in shrinkage ranging from 2 to 18 per cent in brains so fixed, suggested that it would be best to examine this method a little more in detail. Unless uniform results could be obtained by it, this method would, of course, be un-

suited to comparative study of the size of bulb elements. Accordingly, the method was further tested, and at the same time an examination was made of the effect of Müller's fluid and of Orth's Formol-Müller solution upon the various parts of the brain.

A long series of experiments demonstrated quite conclusively the following points which have an important bearing upon the present investigation.

1. Of the three fixing fluids tested, Ohlmacher's solution causes the least change in weight in brain tissue.

2. Orth's solution (cold) causes a slight increase in weight.

3. Müller's solution causes a very considerable increase in the weight of brain tissue as has already been noted (Donaldson, '94).

4. Olfactory bulbs, fixed in Ohlmacher's solution, reach a state of equilibrium at the end of twenty-four hours; fixed in Formol-Müller, they reach this state at about the end of one week; fixed in Müller's solution alone, changes continue from six weeks to two months.

5. There seems to be no appreciable individual variation in the reactions of albino rat brains of like age to Ohlmacher's solution, to Müller's fluid, or to the Formol-Müller solution. The results obtained by Dr. King are due apparently to the fact that the brains which were weighed in her experiments had been fixed for varying short lengths of time and the initial changes in weight were so rapid that there appeared to be a considerable difference in the way the various brains reacted to the fixative, when in reality, had all the brains been fixed for exactly the same length of time, no such large disagreement would have been found.

II. THE PROBLEM OF SIZE DIFFERENCES

Although under normal conditions, there is a good deal of variation in the size of the olfactory bulb of the albino rat, we have found that it is possible, experimentally, to increase this range of variation to a very considerable degree. The question next arises as to the structural cause of the difference

in size. Is it one of size of elements or of their number? Have we more cells and fibers in the heavier bulb or are the cells and fibers merely of a larger size? The present paper deals only with the question of cells. The fibers have yet to be examined.

III. TECHNIQUE AND METHODS OF STUDY

Since experiments on the effect upon the rat brain, of Ohlmacher's, Müller's, and the Formol-Müller solutions have demonstrated that for brains of like ages there is a definite, practically unvaried, swelling or shrinking reaction for any given fluid, the brains of the rats used in the defective diet and exercise experiments were fixed in these several solutions for histological comparison. For the present cell study the method adopted was that recommended by King ('10) for study of the cortex, namely fixation in Ohlmacher's solution for twenty-four hours, followed by one hour in 85 per cent alcohol, three to four days in iodized 70 per cent alcohol, double embedding in celloidin and paraffin, and staining in carbol-thionin and eosin. However, after the first trials, this method was varied in the matter of embedding. For such small objects as the olfactory bulbs, paraffin proved more satisfactory when used alone. Sections were cut 8μ thick and mounted serially. A rather deep thionin stain gave the best results for cell enumeration.

1. Preparation of sections

At first, some bulbs were cut sagittally and the largest sections compared. In the study of these sections the number of cells in the gray layer of the different bulbs was found to be so nearly identical that it was decided to attempt a thorough study of cell number.

In dissecting a rat brain into its parts, the bulbs are cut from the brain in such a way as to leave an appreciable portion of the bulb attached to the cerebrum. The method followed was to place the brain, ventral side down, on a flat surface and with a knife held in a plane perpendicular to the table, to sever the bulb at the point where it disappears beneath the cerebrum (plate 1).

This is the part of the bulb which is weighed, and since all bulbs are removed in the same way, it has been assumed that we have corresponding portions for comparison. Because the recorded weights represented only this portion of the bulbs, it seemed advisable at first to compare the cell elements of these parts only. Accordingly cross sections of the bulbs were made but unfortunately, as appeared later,most of the test series were not complete for the portion of the gray substance beneath the hemispheres. The method of meeting this difficulty will be described later.

2. Methods of study

The study of sections was made largely with the aid of the Edinger projection apparatus. Cell counts were made by projecting the sections onto white wrapping paper, outlining the area, and punching the image of each cell nucleus with a tallying register fitted with a sharp prong in place of the usual blunt register arm (Hardesty '99). The hole punched by this prong insured against counting the same cell twice. It also left a permanent record of any particular region which could be re-examined later. In some cases the count for each section was recorded; in others, the whole number of sections to be counted were registered consecutively and no record made until the end. Occasionally a section was recounted—to serve as a check on the work.

IV. GENERAL DIFFERENCES IN SIZE

Most of the comparisons of size and the determinations of cell number have been made on bulbs stunted by a defective diet, and on their respective controls. Only two bulbs of the exercise series have yet been examined.

The general differences in size between young and mature, stunted and normal olfactory bulbs are very well illustrated by the sections shown in Plates 1, 2, and 3. Figure 1 of plate 1 is a camera drawing of a median sagittal section through the bulb of a rat stunted by feeding for thirty-one days on a corn diet. The body length was 138 mm.; brain weight, 1.547 grams and the weight of the corresponding bulb which was removed and

weighed, was 0.020 gram—the approximate weight then of the bulb shown in the drawing. Figure 2, plate 1, is a median sagittal section through the control bulb. The rat from which this was taken was 166 mm. long, with brain weight of 1.698 grams, and the weight of the corresponding bulb was 0.032 gram. Like the bulbs of very young rats, the gray layer of the stunted bulbs extends somewhat further, in proportion, beneath the cerebrum than in the case of normal older individuals.

When the stunted bulb is compared with its control, there appears to be a rather uniform size difference involving all parts of the bulb. It is hard to compare the outer fiber layers owing to the difficulty in removing the bulbs perfectly from the skull. The anterior end of the fiber layer is very likely to be entirely torn away and sometimes the ventral side also suffers. However, it is plain that the glomeruli of the larger bulb are much larger and more open; the granular cells are not so closely packed together; and the gray layer is usually broader in the larger bulb and the inner granular area considerably more extensive. These differences between the peripheral portions are illustrated by the more highly magnified mid-dorsal areas S and S of figures 1 and 2, shown in figures 3 and 4 of plate 2.

Plate 3 shows three cross sections; through Q_5, a thirty-day control bulb (fig. 5); M_4, a sixty-two-day stunted bulb (fig. 6); and M_5, the sixty-two-day normal control (fig. 7), for M_4. These sections were made through the region where the bulbs are usually cut from the brain. The figures illustrate another typical difference. The normal bulb (figs. 5 and 7), as it grows, elongates more rapidly than it increases in thickness and it tends to grow faster dorso-ventrally rather than laterally. In these figures, the outer fiber layer is probably complete at the sides. Ventrally it has doubtless been torn away to some extent in all three bulbs. The difference in size of the glomeruli is well shown here, but while there is a greater area of gray matter in figure 7 than in the other two, the gray layer seems narrower than in M_4 (fig. 6). The companion bulb of Q_5, (fig. 5) weighed 0.024 gram, that of M_4 (fig. 6), 0.025 gram while M_5 weighed 0.037 gram (fig. 7). The portion of Q_5 anterior to the section

illustrated, was about 1350μ long, while M_4 had 1500μ anterior to the section, and M_5, 2000μ. The differences in size are confined to no one region but are distributed somewhat proportionally through the different layers.

V. COMPARISON OF CELLS OF GRAY LAYER

1. Size and number of small cells in molecular layer

It has been the general impression that, within certain limits, the size and weight of the brain are indices to its functional capacity. In the phylogenetic series, of course, it is, with one or two exceptions, true that increase in intelligence is accompanied by increase in the relative size of the brain. So within any given species of mammals, it has been assumed that the more efficient brain is the larger and heavier.

The question as to whether, within such a group, increase in size of the brain means an increase in the number of nerve elements or in the size of the elements themselves, becomes an important one. For an increase in the number of elements should give increased functional possibilities. So, if we find in comparing large and small brains or divisions of brains from closely related animals, that the larger structure contains a greater number of cells and fibers, then we have reason to expect from the larger and more complex structure the greater degree of efficiency.

If, on the other hand, the number of elements is found to be uniform for the part under consideration, then we must conclude that the large and the small brains have potentially the same ability to function, save as their efficiency may be affected by the size or degree of development of the individual elements.

The small cells of the molecular layer (*mo*, fig. 2) show more cytoplasm; or perhaps we may say that it is possible to distinguish more cells with cytoplasm in the molecular layer of large bulbs than of small ones. For example, the section of F_1 shown in figure 1 shows 68 cells between mitral layer and glomeruli, in which cytoplasm may be distinguished, while the control, F_2, shows 158 such cells. Corresponding sections through M_1,

a thirty-day control, and C_3, a sixty-day underfed bulb, show 108 and 103 cells with cytoplasm.

Although a difference in cell size appeared, there seemed to be little difference in numbers of cell elements in the gray layer. Although it is not always possible to distinguish between the nuclei of very small cells and possible cross sections of fibers under the conditions used for counting—yet the error due to this difficulty is probably negligible. A preliminary count was made of all elements, having the appearance of nuclei in the largest sections of the bulbs F_1, C_3, F_5, F_6, and M_1, with the following results.

TABLE 1

INITIAL CONTROL				TEST				FINAL CONTROL			
Bulb	Age	Bulb weight	Num-ber cells	Bulb	Age	Bulb weight	Num-ber cells	Bulb	Age	Bulb weight	Num-ber cells
	days	*grams*			*days*	*grams*			*days*	*grams*	
M_1....	30	0.029	2172	F_1	62	0.020	2569	F_5	61	0.032	2569
				C_3	59	0.021	2604	F_6	61	0.033	2693

These counts for the test and final control bulbs suggested so strongly that the number of cells is the same for bulbs of different sizes that attention was turned entirely to the investigation of this point. At first longitudinal sections were used, but these were soon abandoned for two reasons. First, it seemed desirable to be able to count the cells of just that portion of the bulbs corresponding to the part weighed; and second, the longitudinal sections presented so many irregularities that it was necessary to count many more sections to approximate the true average than in the case of the cross sections. Counts were made of all elements in the gray layer outside the mitral layer between the tip of the bulb and the point at the proximal end where the gray layer is first interrupted on the dorsal aspect of the bulb (see figs. 5, 6, 7). These counts consumed a vast amount of time and when completed seemed to disagree with the observations already made upon the longitudinal sections (table 2).

A first glance at the table would indicate that the small bulb has fewer cells and would suggest that this difference in cell

TABLE 2

BULB	AGE	HISTORY	BRAIN WEIGHT	WEIGHT 1 BULB	NUMBER SMALL CELLS
	days		*grams*	*grams*	
X_1....	30	Control	1.338	0.017	636,656
M_7....	62	Underfed 31 days	1.461	0.025	662,982
G_{12}....	62	Underfed 31 days	1.543	0.027	601,982
G_6.....	61	Normal control	1.630	0.031	675,305
M_5....	62	Normal control	1.711	0.037	716,582
X_6....	134	Revolving cage 104 days	1.927	0.040	789,680

number is one of the factors in bulb size. But corresponding sagittal sections had given fairly close agreement in numbers and the study of sagittal sections made it more and more evident that these counts of cross sections could be taken only to compare the parts commonly considered the bulb and not for an enumeration of the cells in the whole bulb. The difference in shape in the large and small bulbs made it apparent that a true count must be made either from sagittal sections or from cross sections cut through the entire length of the gray matter covering the bulb. Comparison of such sections as figures 1 and 2 made it clear that if we had, in reality, a constant number of cells in the gray layer, the numbers in the regions here designated as the 'bulb' could scarcely be expected to show any closer agreement than we find in this table, and would probably have the relations there given. For the larger and better developed the bulb, the greater the proportion of it lying anterior to the cerebrum, while the young or the stunted bulb runs somewhat further back beneath the hemisphere and so some of the cells escaped enumeration. For example, M_5, a bulb of 0.037 gram, has 271 sections containing mitral cells in the portion of the bulb beneath the cerebrum. M_4, the test bulb of this litter, which weighed but 0.025 gram had 336 sections in this region. Taking these facts into consideration, the table in question pointed to a uniformity rather than variation in numbers corresponding to size. Later we shall see how, in the light of the study of the mitral layer, a part of this table can be shown to closely conform to this supposition that the number of cells in the entire gray layer is approximately constant for olfactory bulbs of different sizes.

2. Size and number of mitral cells

The cells of the mitral layer show a good deal of variation in size and shape and there is much difference in these respects in different regions of the same bulb. This makes the comparison of the size of the mitral cells in large and small bulbs rather difficult.

But if all the mitral cells of a section from a small bulb are drawn with a high magnification by means of camera lucida or projection apparatus and those cells arranged side by side with a series from a corresponding section of a large bulb, drawn to the same scale, it is possible to make a general comparison. In this way the mitral cells have been compared, and there is no doubt I think that the mitral cells of large bulbs are larger and better developed than those of small bulbs.

Details of technique and examination. It was sometimes quite difficult to determine whether a cell should be counted or not. For instance, when counting mitral cells it was hard to know at times whether a cell was a mitral cell or a brush cell, as many cells occur in the mitral layer which are exactly like those large cells occurring in the molecular layer but which lack the typical mitral form. On the other hand, typical mitral cells occur not infrequently out in the molecular layer or even among the granules on the inner edge of the glomerular layer. For this reason and in order that there might not be any unconscious influence in deciding whether cells should be counted, an attempt was made to vary the order of procedure for each successive count.

A rather complete count was made of the mitral cells of fourteen bulbs and of the small cells of the gray layer in four bulbs. Eight of these were cut longitudinally and six cut transversely.

The first series counted were those of X_1, Initial control, Series E and X_6, Test, Revolving Cage Series. In both series every other section was counted for the region anterior to the cerebrum—corresponding to the portions of these same bulbs in which the small cells of the gray layer had been counted. The result was 64,470 cells for X_1, a 0.017 gram. thirty-day control

bulb. The number for X_6, whose weight was 0.040 gram, was 73,950. To see whether there were any virtue in making so thorough a count of cross sections, the total number was computed from a recount of every 10th section, excepting at the most anterior end where every cell was counted in every section, until the sections showed a single layer of mitral cells. By this method the number obtained for X_1 was 64,775 cells, making a difference of only 0.4 per cent. For X_6 the count was 73,324, which was 0.8 per cent smaller than the more exact count obtained by counting half the sections. These differences were so small as to make the more exhaustive count seem unnecessary. X_6 gave an almost complete series through the entire gray layer so the count was completed, giving for the entire bulb 80,114 cells. The count of the mitral cells in X_1 could not be completed as the series had been cut, unfortunately, with the idea of comparing only the parts of the bulbs whose weights we knew, and which, therefore, extended back but a short distance under the cerebrum. The cells of these few sections were, however, counted, giving a total of 71,914.

The number of mitral cells in G_6 was computed from absolute counts of anterior and posterior ends of the series and by counting every tenth section through the rest of the series. M_4, M_5 and Q_5 ran so evenly that here in the middle portion of each series, only every twentieth section was counted; on either side of this portion, every tenth section, and all cells of all sections at either end.

With the sagittal sections, the task was more difficult and the results, I believe, less reliable for this reason: toward the sides of the bulbs, especially the median side, the sagittal series may give tangential sections of the mitral layer so that a single section may yield a count of 1500 cells whereas a section two or three removed on either side might have but 300 or so mitral cells. It can be easily seen that if the section to be counted, happened to fall in such a region, or entirely skipped such a region, the count would be considerably modified. Some of the bulbs gave no trouble of this kind while others were hard to count for this reason. G_3 was an interesting example of the way this

may work out.   A count was first made of all cells at either end of the series and those in every tenth section through the middle portion.   The result when computed was 95,993 mitral cells. Then the middle section of every ten was counted with a total result of 83,974 cells.   Two other series were attempted but abandoned as the bulbs were so irregular that an accurate count would have required the enumeration of the cells of at least every alternate section.   The other bulbs, except C_3 for which every fifth section was counted, were fairly regular so that the mitral layer offered no such complications.   For these, the method of counting all cells at either end of the series and those of every tenth section through the median portion was followed.   The sequence of counts was varied with each bulb, and the records kept in various ways and not infrequent recounts made.   The recounts were surprisingly close to the original, for, as has been stated, it is not always easy to decide whether or not a cell should be counted, and in focusing as one counts, a granule lying below or above a portion of a mitral cell sometimes looks very like a nucleus, but the error due to this cause is probably too small to be considered.

Details of counts of the different bulbs, arranged in the order followed in table 3.

Bulb X_1, thirty day, initial control.   Cross sections.   Mitral cells counted in every section of anterior end back to the first section in which the mitral cells appeared in a single layer.   From this point, counts were made for every tenth section back to the cerebrum.   By computation, the total number of mitral cells was 64,775.   By a recount in which the mitral cells of every other section were enumerated the computed number was 64,470 making a difference of only 0.4 per cent in the two counts.   The series of sections for the region beneath the cerebrum was incomplete, the posterior portion not having been preserved.   A count was made, however, of the sections which were present.   This number added to the number already counted by the second method, brought the total up to 71,914 cells.

Bulb E_1, Test, defective diet series.   Sixty-two days.   Sagittal sections.   Mitral cells counted in all sections at either end of the series and for every tenth section between.

Bulb C_3 Test, defective diet series.   Fifty-nine days.   Sagittal sections.   Counts made as in E_1.

Bulb Q_5, thirty day, initial control.   Defective diet series.   Cross sections.   Mitral cells counted in all sections at both ends of the series.

TABLE 3

Giving number of mitral cells in one olfactory bulb of the albino rat

Arranged according to bulb weight

RAT	AGE	BODY LENGTH	BRAIN WEIGHT	WEIGHT 1 BULB	NO. MITRAL CELLS	REMARKS
	days	mm.	grams	grams		
X₁ Control.....	30	101	1.338	0.017	71,914	Very incomplete. Up to point of union with cerebellum 64,470 cells, cross section
E₁ Test........	62	138	1.547	0.020	71,527	Sagittal section
C₃ Test........	59	142	1.482	0.022	79,165	Sagittal section
Q₅ Control.....	30	97	1.316	0.024	82,192	Probably about 300 more cells. Cross section
C₅ Test........	59	150	1.578	0.024	70,625	Sagittal section
M₄ Test........	62	109	1.461	0.025	76,611	Cross section
G₃ Test........	61	136	1.456	0.027	83,974	Sagittal section
O₅ Test........	115	121	1.556	0.029	71,663	Sagittal section
G₆ Control.....	61	166	1.630	0.031	81,638	Cross section
F₅ Control.....	61	166	1.698	0.032	71,468	Sagittal section
C₇ Control.....	62	174	1.709	0.036	79,839	Sagittal section
M₅ Control....	62	169	1.711	0.037	76,596	Cross section
X₆ R. C. Test..	134	193	1.927	0.040	80,114	Up to point of union with cerebrum 73,950 cells, cross section
O₁ Control.....	115	175	1.792	0.041	72,333	Sagittal section (X₁ omitted from average)
Average.....					76,749	

Standard deviation $\sigma = 4564$

Probable error of the mean ± 855

Through the middle region only every twentieth section was counted as the sections were extremely uniform. Through the two regions between this middle portion and the ends in which all cells were counted the mitral cells for every tenth section were counted.

Bulb C_5, test, defective diet series. Fifty-nine days. Sagittal sections. Mitral cells counted for all sections at both ends of the series and every fifth section of the rest of the series.

Bulb M_4, test, defective diet series. Sixty-two days. Cross sections. Mitral cells were counted as in Q_5. Count was made also of all cell elements in every alternate section in the gray layer back to the anterior end of the cerebrum. Computation was made for entire series.

Bulb G_3, test, defective diet series. Sixty-one days. Sagittal sections. Mitral cells were counted for all sections at ends of series and for every tenth between. The computed result was 95,993. The middle sections between every tenth were then counted, giving a

TABLE 4

Giving number of mitral cells in one olfactory bulb of the albino rat

Arranged by litters

AGE	RAT	WEIGHT 1 BULB	NUMBER MITRAL CELLS	PERCENTAGE DIFFERENCE OF TEST	REMARKS
days		*grams*			
62	F_1 Test	0.020	71,527 (S.)	+ 0.08	
62	F_5 Control	0.032	71,468 (S.)		
115	O_5 Test	0.029	71,663 (S.)	− 0.9	
115	O_1 Control	0.041	72,333 (S.)		
62	M_4 Test	0.025	76,611 (C.)	+ 0.2	
62	M_5 Control	0.037	76,596 (C.)		
59	C_3 Test	0.022	79,165 (S.)	− 0.9 ⎱ Ave.	
59	C_5 Test	0.024	70,625 (S.)	−12.0 ⎰ −6.5	
62	C_7 Control	0.036	79,839 (S.)		
30	X_1 30 d. T.	0.017	71,914 (C.)		Very incomplete
134	X_6 R. C. T.	0.040	80,114 (C.)		Slightly incomplete (not in average)
61	G_3 Test	0.027	83,974 (S.)	+ 2.9	
61	G_6 Control	0.031	81,638 (C.)		
30	Q_5 30 d. C.	0.024	82,192 (C.)		Probably 300 more cells
Average per cent difference of test....				− 1.8	

(S.) = Sagittal section.

(C.) = Cross section.

count for every fifth section of this region. The computation then gave a total of 83,974. Two other bulbs of this litter were also cut in sagittal sections and an attempt was made to count the mitral cells but the bulbs were so irregular that it would have been necessary to count practically every section, so these counts were abandoned.

Bulb O_5, Test, defective diet series. One hundred and fifteen days. Sagittal section. Counts made as in E_1.

Bulb G_6, control, defective diet series. Sixty-one days. Cross section. Counts made as in E_1.

Bulb F_5, control, defective diet series. Sixty-one days. Cross section. Counts made as in E_1.

Bulb C_7, control, defective diet series. Sixty-two days. Sagittal sections. Counts as in E_1.

Bulb M_5, control, defective diet series. Sixty-two days. Cross sections. Counts as in M_4 and computation of all cell elements in gray layer made for entire series.

Bulb X_6, test, revolving-cage series. One hundred and thirty-four days. Cross sections. Mitral cells counted by both methods described for X_1. Also all cell elements of the gray layer computed for the entire series as in M_4 and M_5.

Bulb O_1, control, defective diet series. One hundred and fifteen days. Sagittal section. Mitral cells counted as in E_1.

Table 3 gives the results of the counts of the mitral cells of fourteen olfactory bulbs, arranged according to bulb weight. It is obvious that there is no correlation between bulb size and the number of the mitral cells or between age—within the limits taken—and number of cells. The numbers range from 70,625 to 83,974 with an average of 76,750 cells for 13 bulbs, X_1 being omitted from the average. I am inclined to think 83,974 cells is too high a count for G_3 and that still closer enumeration might yield a lower number. A recount was made for C_5 counting every fifth section, as this was a somewhat irregular bulb and it was thought that might account for the variation of this bulb from the rest of the litter. But the recount gave practically the original number.

Table 4 which is arranged by litters indicates a striking agreement between the members of the same litter. With the exception of litter C, in which C_5 falls 12 per cent below the control in number of mitral cells, there is extremely little difference between test and control bulbs of the same litter. So we find that whether the bulb has been stunted by a defective diet, or enlarged by exercise, the number of mitral cells is practically constant for any given litter. The factors which have brought about a change in size of the olfactory bulbs have failed to affect the number of mitral cells, at least in the gray layer. The test and control counts are, with one exception, extremely close. This is a fresh example of the similarity in structure among members of the same litter—a relation which is continually appearing in the study of this animal.

3. Study of the small cells in the gray layer

The sections of bulbs M_4, M_5, X_1, and X_6 are all series in which a count was made of the small cells of the gray layer in the anterior portion of the bulb as well as of the mitral cells. Assuming that the relation between the number of mitral cells in two given sections of a bulb would be the same as that between the small cells of the gray layer in these same regions, computation was made of the total number of small cells in the gray layer of M_4,

M_5, and X_6. Bulb X_1 was too incomplete to make such calculation possible. Let us take M_4. The small cells were counted back to section 4, 1/1. We have the total number of mitral cells and also the number of mitral cells back to section 4, 1/1. This gives us the data for computing the total number of small cells as follows:

Mitral cells of M_4 to section 4, 1 1.............................. 50,729
Total mitral cells.. 76,611
66 per cent of mitral cells in anterior portion.
Number small cells to section 4, 1/1............................ 662,982
If this number equals 66 per cent of the total number, then the total number of small cells in the gray layer would be 1,004,518.
If we treat M_5 in the same way we have:
Mitral cells to section 3, 6 12........ 55,775
Total mitral cells.................... 76,595
73 per cent of mitral cells in anterior portion.
Number small cells to section 3, 6/12...........................716,382
Then total number small cells..................................981,619
According to this computation the test bulb would have two per cent more cells than the control.
Now if we treat X_6 in like manner we have the following:
Mitral cells to section 5, 1 7........... 62,060
Total mitral cells............... 80,114
77 per cent of mitral cells in anterior portion.
Small cells to section 5, 1/7........ 789,680
Then total number of small cells...... ....1,020 258

These total numbers are strikingly close. The number of bulbs is too small to warrant us in drawing general conclusions but I think the results certainly point to close agreement in number even of the small cell elements in the gray layer.

While there is a constant increase in the number of myelinated fibers correlated with age, as has been demonstrated by Greenman ('13) for the peroneal nerve, Boughton ('06) for the oculomotor, Hatai ('02) and ('03) for both dorsal and ventral roots of several spinal nerves, and Dunn ('12) for the ventral root of the second cervical nerve; there is very little evidence of any true increase in the number of cells in the central nervous system after the first few days after birth. Allen ('12) found dividing cells in the cerebellum up to twenty-five days and in the cerebrum up to twenty days, with a few along the lateral walls of

the lateral ventricles until the end of the second year. Hatai observed an increase in number of cells in the spinal ganglia, corresponding to increase in age but this increase was attributed in part, at least, to failure to count all the ganglion cells in very small animals. Ranson ('06) in a study of the second cervical nerve found no correlation between the number of cells and the number of myelinated fibers, neither did he find the number of cells to vary with the age of the rat.

The results of the present investigation of the number of cells in the olfactory bulb help to confirm the impression that the number of cells in the central nervous system becomes fixed at an early age so that after the first three or four weeks at least, there is no material change in the cell number.

This study also gives us reason to believe that the number of small and of mitral cells in the gray layer of the olfactory bulb is very nearly the same for all individuals with especially close agreement between individuals of the same litter. It seems fairly evident that while external conditions may modify to a considerable extent the size of the brain of the albino rat and especially the size of the olfactory bulbs, the only effect is upon the relative development of the individual cells. The number of cells remains the same. The fibers have yet to be examined.

It is important to bear in mind in a determination of this sort—e.g., the number of mitral cells—that a fixed number, in the physical sense, is not to be expected, for all organisms are normally variable in all of their parts, variability being an essential character for living things; so the number which is obtained gives a mean value which we take to be characteristic for the species under the present conditions, but around which equally characteristic variations also occur.

VI. CONCLUSIONS

1. For bulbs of different ages and sizes, the regions anterior to the cerebrum, which are commonly considered the bulbs, are not strictly homologous, since, in the brains of young or stunted rats, a larger proportion of the bulb lies beneath the cerebrum than in the case of the better developed brains.

2. All layers of the olfactory bulb are about equally concerned in the increase in size or in the arrest of development of the bulb.

3. The small cells of the molecular layer show a larger amount of cytoplasm in large bulbs than in small ones.

4. The number of small cells in the molecular layer, apparently, is not correlated either with age of the rat or size of the bulb. The entire computed number for a small, medium, and large bulb was found to be approximately 1,000,000 cells ± 2 per cent.

5. The mitral cells of small bulbs are smaller, on the average, than those of large bulbs.

6. Within the limits here taken the number of mitral cells is not affected by the age or the size of the bulb.

7. There seems to be some variation between litters in the number of the mitral cells. The average number of mitral cells for 13 bulbs was 76,750, the lowest number being 70,625, and the highest 83,974. The standard deviation σ is 4564 and the probable error of the mean ± 855.

8. When members of the same litter are compared, bulbs stunted by a defective diet or enlarged by exercise show practically the same number of mitral cells as do their controls. The mean difference is −1.8 per cent for the tests.

9. The olfactory bulb size is correlated with cell size and not with cell number.

VII. LITERATURE CITED

ALLEN, E. 1912 The cessation of mitosis in the central nervous system of the albino rat. Jour. Comp. Neur., vol. 22, no. 6, pp. 547–568.

BOUGHTON, T. H. 1906 The increase in the number and size of the medullated fibers in the oculomotor nerve of the white rat and of the cat at different ages. Jour. Comp. Neur., vol. 16, pp. 153–165.

DONALDSON, H. H. 1894 Preliminary observations on some changes caused in the nervous tissues by reagents commonly employed to harden them. Jour. Morph., vol. 9, pp. 123–166.

DUNN, E. H. 1912 The influence of age, sex, weight, and relationship upon the number of medullated nerve fibers and on the size of the largest fibers in the ventral root of the second cervical nerve of the albino rat. Jour. Comp. Neur., vol. 22, pp. 131–157.

GREENMAN, M. J. 1913 Studies on the regeneration of the peroneal nerve of the albino rat: number and sectional areas of fibers: area relation of axis to sheath. Jour. Comp. Neur., vol. 23, no. 5, pp. 479–513.

HARDESTY, I. 1899 The number and arrangement of the fibers forming the spinal nerves of the frog (Rana virescens). Jour. Comp. Neur., vol. 9, no. 1, p. 104.

HATAI, S. 1902 Number and size of the spinal ganglion cells and dorsal root fibers in the white rat at different ages. Jour. Comp. Neur., vol. 12, pp. 107–124.
1903 On the increase in the number of medullated nerve fibers in the ventral roots of the spinal nerves of the growing white rat. Jour. Comp. Neur., vol. 13, pp. 177–183.

HOLT, C. M. 1917 Studies on the olfactory bulbs of the albino rat. I. Experiments to determine the effect of a defective diet and of exercise upon the weight of the olfactory bulbs. Jour. Comp. Neur., vol. 17, pp. 201–234.

KING, H. D. 1910 The effects of various fixatives on the brain of the albino rat, with an account of a method of preparing this material for a study of the cells in the cortex. Anat. Rec., vol. 4, pp. 214–244.

RANSON, S. W. 1906 Retrograde degeneration in the spinal nerves. Jour. Comp. Neur., vol. 16, pp. 3–31.

ABBREVIATIONS

s, areas in figures 1 and 2 enlarged in figures 3 and 4.
fi, outer fiber layer
gl, glomeruli
mo, molecular layer

mi, mitral layer
g, granular layer
f, inner fiber layer
c, cerebrum

PLATE 1

EXPLANATION OF FIGURES

Median longitudinal section through olfactory bulb of F_1, section 3 1/6. F_1, underfed 31 days. Final brain weight, 1.5470 grams, bulb weight, 0.0203 gram. Defective diet. Magnified 24 diameters.

Median longitudinal section through olfactory bulb of F_5, section 5 5/4. F_5, control for F_1. Brain weight, 1.6984 grams, bulb weight, 0.0315 gram. Magnified 24 diameters.
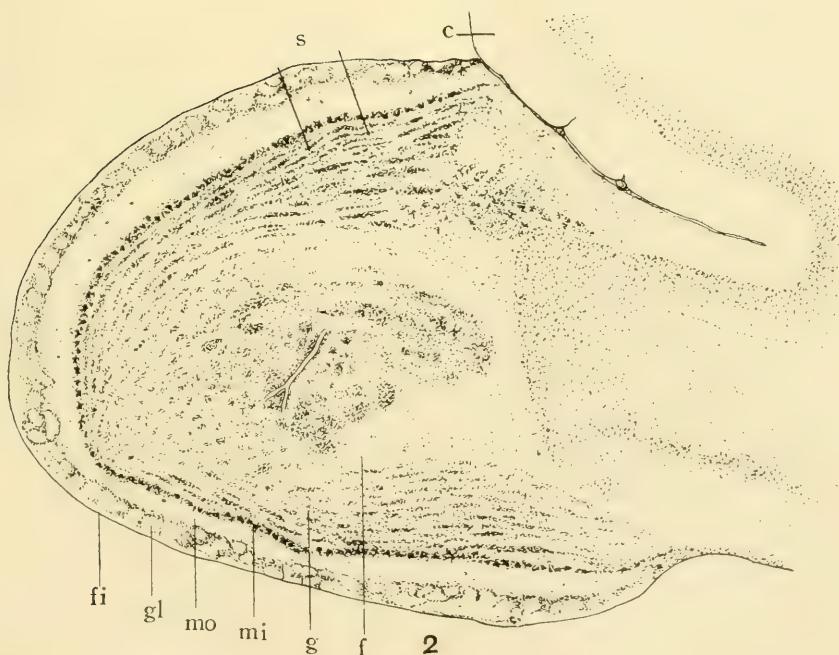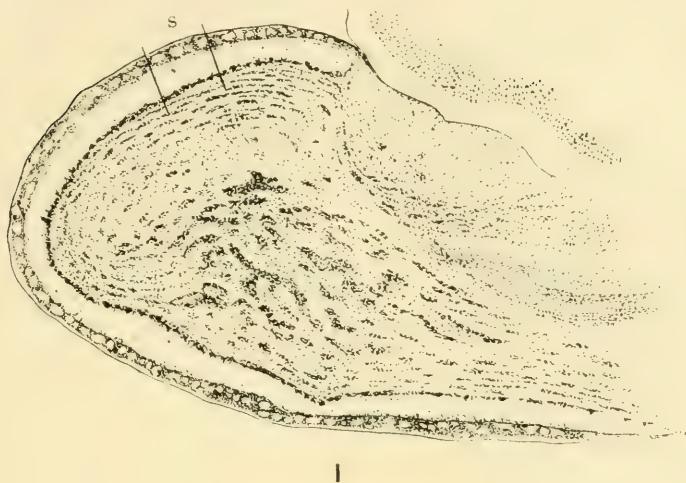
s

1

s   c

fi   gl   mo   mi   g   f   2

3

4
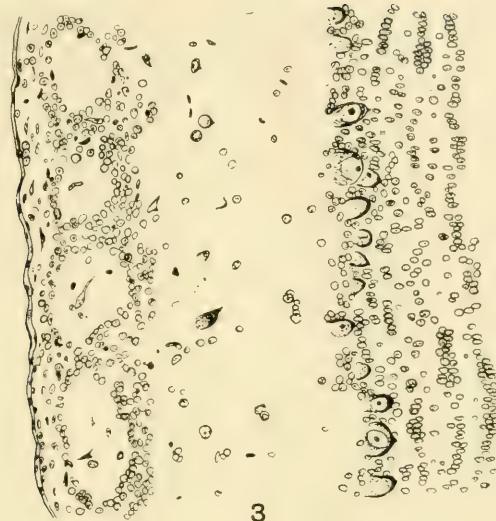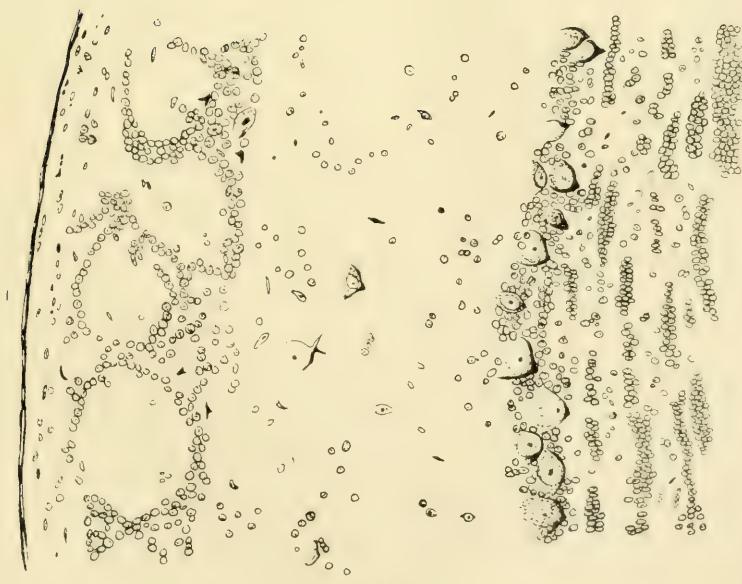
Cross section of Q_5, section 2 3/3, cut at region where bulb joins cerebrum. Q_5, 30-day control rat, killed when weaned. Brain weight, 1.3164 gram; bulb weight, 0.0238 gram. Magnified 30 diameters.

Cross section of M_4, section 4 1/1, cut same region as figure 5 above. M_4, underfed 30 days. Brain weight, 1.4613 grams; bulb weight, 0.0251 gram. Defective diet. Magnified 30 diameters.

5

6
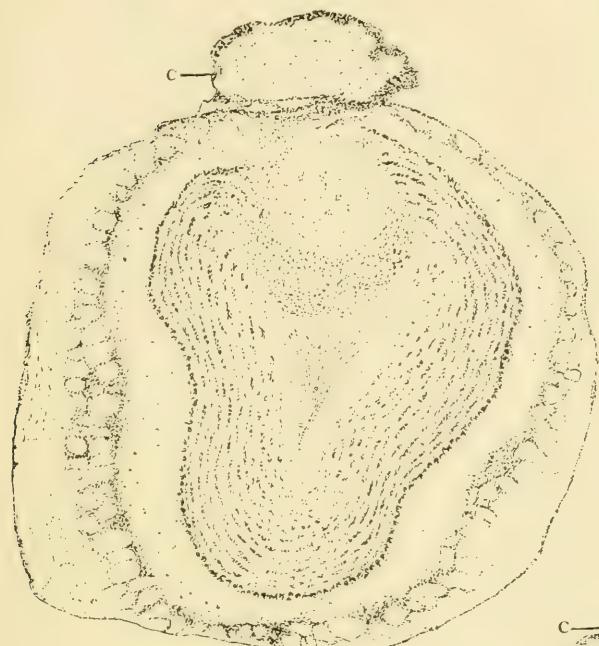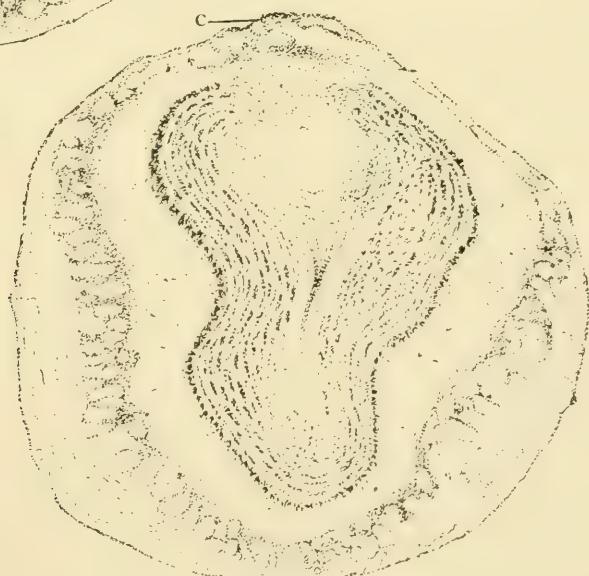
Cross section of M_5, section 3 6/12, cut same as figure 5 above. M_5, control for M_4. Brain weight, 1.7110 grams; bulb weight, 0.0374 gram. Magnified 30 diameters.

7
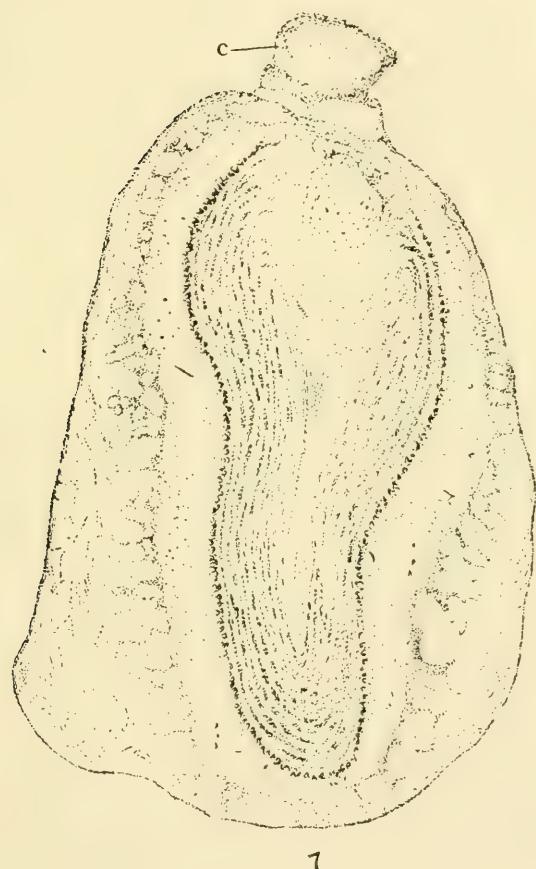
[Dedicated to the memory of my friend Mrs. Susanna Phelps Gage]

FURTHER CONTRIBUTIONS ON NEUROBIOTAXIS

IX. AN ATTEMPT TO COMPARE THE PHENOMENA OF NEUROBIOTAXIS WITH OTHER PHENOMENA OF TAXIS AND TROPISM. THE DYNAMIC POLARIZATION OF THE NEURONE

C. U. ARIËNS KAPPERS

From The Central Institute for Brain Research, Amsterdam

SIX FIGURES

CONTENTS

NEUROBIOTAXIS AND ITS SELECTIVE CHARACTER

In various articles,[1] first in 1907, I have published observations concerning the shifting of nerve cells in the central nervous system, which could be shown by the different places that

[1] The principal points are mentioned in:

Die phylogenetische Verlagerungen der motorischen Oblongata-Kerne, ihre Ursache und ihre Bedeutung. Neurologisches Centralblatt, 1907, and Rapport du Congrès international de Psychiatrie et de Neurologie, Amsterdam, 1907.

Weitere Mitteilungen über die Verlagerungen der motorischen Oblongata-Kerne: der Bau des autonomen Systems. Folia Neurobiologica, Bd. 1, 1908.

Specially in: Weitere Mitteilungen über Neurobiotaxis. Die Selektivität der

261

the motor nuclei of the oblongata exhibit in the series of vertebrates.

Ontogenetically the same phenomenon could be stated. Since it was most evident that the shifting of these central groups took place in the direction of the point whence the majority of stimuli proceeded to their cells, we apparently had to do with a phenomenon of taxis or tropism, which I called Neurobiotaxis, because it occurs in the nervous system during life (in its phylogenetic and ontogenetic development) and I did not know where

Zellen-Wanderung. Die Bedeutung synchronischer Reizverwandtschaft, etc. Folia Neurobiologica, Bd. 1, 1908.

Über die Bildung von Faserverbindungen auf Grund von simultanen und sukzessiven Reizen. Bericht des III. Kongresses für experimentelle Psychologie in Frankfort am Main, 1908.

Further anatomical details are found in:

Weitere Mitteilungen über Neurobiotaxis, II. Die phylogenetische Entwicklung des horizontalen Schenkels des Facialiswurzelknies. Folia Neurobiologica, Bd. 2, 1908.

Weitere Mitteilungen über Neurobiotaxis, III. Über den Einfluss der Neurone der Geschmackskerne auf den motorischen Facialis und Glossopharyngeuskern und ihr Verhalten zur Radix descendens Nervi quinti. Folia Neurobiologica, Bd. 3, 1909.

Weitere Mitteilungen über Neurobiotaxis, IV. The migrations of the abducens nucleus and the concomitating changes of its root-fibers. Psychiatrische en Neurologische Bladen, Amsterdam, 1910.

The migrations of the motor cells of the bulbar Trigeminus, Abducens and Facialis in the series of vertebrates and the differences in the course of their root-fibers (counted as Mitteilung V.). Verhandelingen der Kon. Akad. v. Wetenschappen, Amsterdam. Tweede Sectie, Deel 16, Nr. 4, 1910.

Weitere Mitteilungen über Neurobiotaxis, VI. The migrations of the motor root-cells of the vagus group and the phylogenetic differentiation of the hypoglossus nucleus from the spino-occipital system. Psychiatrische en Neurologische Bladen, Amsterdam, 1911.

Weitere Mitteilungen über Neurobiotaxis, VII. Die phylogenetische Entwicklung der motorischen Wurzelkerne in Oblongata und Mittelhirn. Folia Neurobiologica, Bd. 6, Sommergängzungsheft, 1912.

Weitere Mitteilungen über Neurobiotaxis, VIII. Über den motorischen Facialis und Glossopharyngeus Wurzel bei niederen Vertebraten. Folia Neurobiologica, Bd. 9, 1912.

The structure of the autonomic nervous system compared with its functional activity. Journal of Physiology (England), vol. 37, 1908, p. 139.

Phenomena of neurobiotaxis in the central nervous system. Section Anatomy and Embryology, of the XVIIth International Congress of Medicine, London, 1913.

to classify it under the phenomena of galvanotaxis, chemo-
taxis or other processes of taxis or tropism known at that time.

This phenomenon of shifting is clearly shown by figures 1 and
2, where the dorsal position of the abducens nucleus in the
shark with its huge fasciculus longitudinalis posterior (*f.l.p.*,
fig. 1) strongly contrasts with the ventral position of the same
nucleus in a bony fish (fig. 2), where the fasciculus longitudinalis
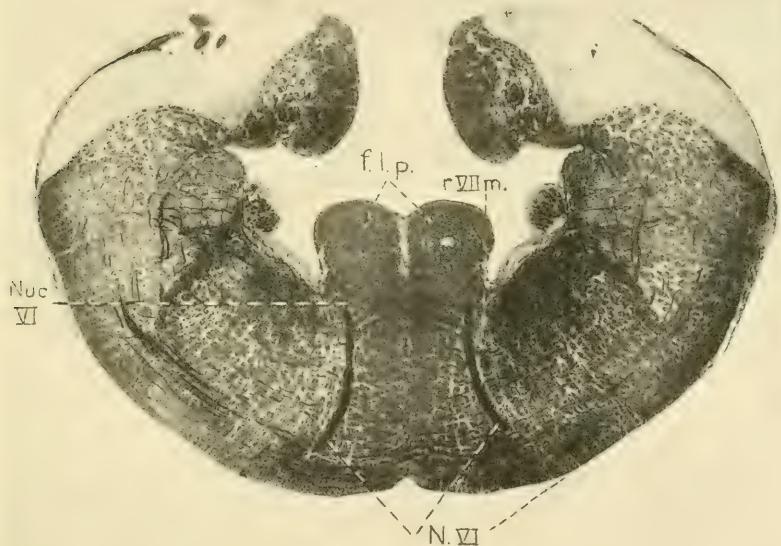
Fig. 1 Acanthias vulgaris, showing the dorsal position of the abducens
nucleus. *f.l.p.*, fasciculus longitudinalis posterior; *Nuc.VI*, abducens nucleus;
N.VI, abducens nerve; *r.VII,m.*, motor facialis root. After Van der Horst.

posterior is much smaller, but where the ventral set of central
afferent tracts which influences this cell group is much more
strongly developed (tr. tecto-bulbares ventrales, *tr.t.b.v.*, fig. 2).

The first way in which I formulated this law was thus: When
from different places stimuli proceed to a cell, its chief dendrite
grows out and its cell-body shifts in the direction whence the

majority of stimuli proceed.[2] The truth of this was soon confirmed also in other parts of the cerebrum, by Tretjakoff,[3] Herrick,[4] Bartelmez,[5] Obenchain,[6] Bok, Van der Horst[7] and others.

I observed, however, on an increase of afferent stimuli in a given center, that not all the neighboring cells approach this center, but that only certain cells proceed to that center which apparently had a certain relation to it, while other cells (even lying nearer by) did not migrate into the direction of the increased sensory field, because evidently they had nothing to do with it and did not stand in relation to it.

Further researches convinced me that the functional relation which appeared to be the condition for the approach was shown to be a correlation depending on simultaneity of function—of stimulation.

So the abducens nucleus shifts from one center of visual co-ordination fibers (the *f.l.p.*) to another set of visual co-ordination fibers (the tr. tecto-bulbaris) if the latter increase; but an increase of the taste fibers for instance, does not have any effect upon it.

[2] Later I found that a similar observation had been already made by Strasser ('92) and by Cajal ('99). Compare: Strasser, Alte und neue Probleme der Entwicklungsgeschichtlichen Forschung auf dem Gebiete des Nervensystems. Ergebnisse der Anatomie und Entwicklungsgeschichte, Bd. 1, 1892, p. 721. Cajal, Textura del sistema nerviosa del hombre y de los vertebrados, vol. I, 1899, p. 560. See also Cajal, Algunas observacionas favorables a la teoria neurotropica. Trabajos, vol. 7, 1908, p. 63. Both, however, failed to see the correlative character in this process, and Cajal ascribes a great influence to the spongioblasts (ependyma and glia) in the secretion of attracting chemicals for the axones, in which I do not at all agree with him.

[3] Tretjakoff. Das Nervensystem von Ammocoetes, 11. Das Gehirn. Archiv f. mikrosk. Anat., Bd. 75, 1909.

[4] Herrick. The morphology of the forebrain in Amphibia and Reptilia. Jour. Comp. Neur., vol. 20, 1910.

[5] Bartelmez. Mauthner's cell and the nucleus motorius tegmenti. Jour. Comp. Neur. vol. 25, 1915.

[6] Obenchain (with Herrick). Notes on the anatomy of a cyclostome brain, Ichthyomyzon concolor. Jour. Comp. Neur. vol. 23, 1913.

[7] Van der Horst. De motorische kernen en banen in de hersenen der visschen, hare taxonomische waarde en neurobiotactische beteekenis. See also: Tijdschrift der Ned. Dierk. Vereen. 1917.

Then I found—though not starting my work with a psychological scope—that the anatomical relations of the dendrites and the cells in the nervous system were regulated in accordance with the law which, in psychology, is known as the law of association, in which law (in all the different forms[8] in which it may appear) the simultaneity of stimulations or residua of stimulations is the essential part.
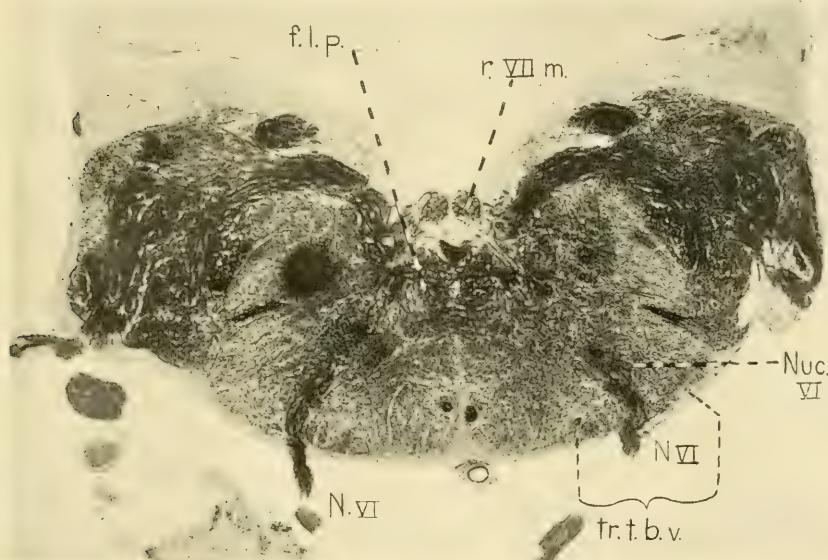
Fig. 2 Tetrodon, showing the ventral position of the abducens nucleus. *f.l.p.*, fasciculus longitudinalis posterior; *Nuc.VI*, abducens nucleus; *N.VI*, abducens nerve; *r.VII,m.*, motor facialis root; *tr.t.b.v.*, tractus tecto-bulbaris ventralis. After Van der Horst.

This anatomical observation, first made on motor cells, led me to study more carefully the courses of several axon-tracts, sensory tracts, as well as the so-called "central motor tracts," such as the pyramids, and it soon appeared to me that a criti-

[8] Those forms are simultaneity, successivity, similarity and contrast. In the three first named forms the presence of one stimulus, or remains of a stimulus, while the other is added, is obvious. The association by contrast is also due in the first place to simultaneity of impression since the simultaneous or successive contrast makes us discriminate things: black and white, father and mother, etc.

cal study of their relation showed most clearly that the same law of neurobiotaxis, the simultaneous relationship in their stimulative function, had been the cause of their final arrangement.[9] So I was able to formulate the phenomena of neurobiotaxis in the following words:

I. If in the nervous system several stimulation-charges occur, the growth of the chief dendrite, and eventually the displace-

A

B
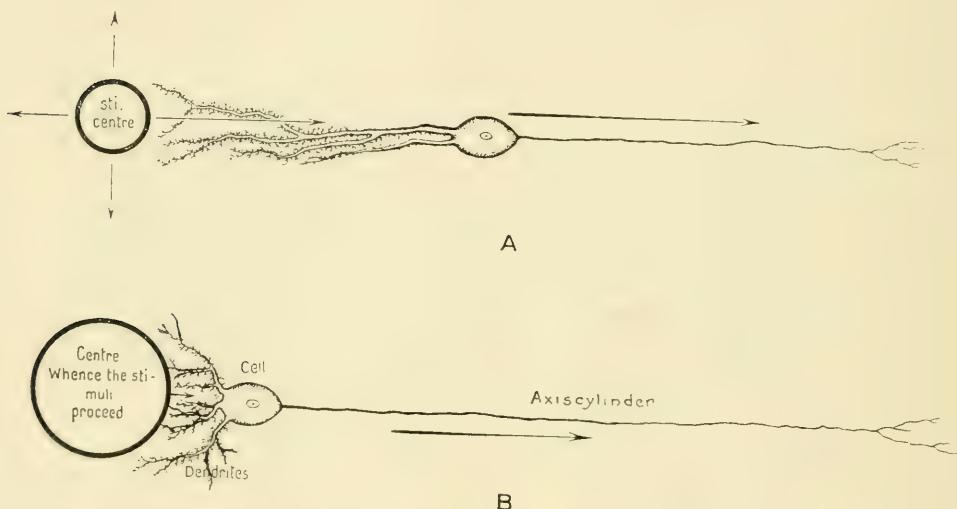
Fig. 3 Showing that, while the axis-cylinder runs with the direction of the nervous current, the dendritic outgrowth and the final shifting of the cell body occur against the nervous current. A, giant dendrites grown out towards the center of stimulation. B, the cell body (perikaryon) has shifted toward the center of stimulation; the axis-cylinder is consequently elongated.

ment of the cell-body itself, takes place in the direction, whence the majority of stimuli proceed to the cell.

II. Only between correlated centers does this outgrowth or shifting take place.

III. The growth of the axis-cylinder (i.e., its final connection) is not primarily regulated by motor centers,[10] but also here synchronic or successive stimulation (correlation) acts a part.[11]

[9] Folia Neurobiologica, Bd. 1, 1908.
[10] Not by some undefined transcendental willing (teleologically).
[11] That is, it is defined by correlation.

While, however, it was evident that the approach of the dendrites and nerve cells to a territory (fig. 3) took place towards the center of the stimulation (as a stimulopetal or centripetal tropism), that is, against the nervous current of stimulation proceeding from this center, the problem became much more difficult to explain how the connection between correlated centers was effected by the axis-cylinder, since it was obvious that the axis-cylinder does not grow towards the stimulation (stimulopetal) to meet it, but moves in the same direction as the stimulus-irradiation (stimulo-fugal or centri-fugal).

BOK'S RESEARCHES: THE STIMULOGENOUS FORMATION OF THE AXON

That the axis-cylinder really grows with the current and that the irradiation of this current plays an important part in its growth has been proved and very carefully examined in this Institute by S. T. Bok, who got highly important results.

Bok[12] found that when an axis-cylinder or a bundle of amyelinated nerve-fibers grows out and passes nerve cells on its way, these nerve cells can be activated to send out an axis-cylinder of themselves in a region perpendicular to the activating axon or bundle (fig. 4).

This fact was found with the fasciculus longitudinalis posterior in such a form as left no doubt, since it appeared that the motor nuclei which undergo the influence of this bundle were only activated according to the degree in which the fasciculus longitudinalis posterior had grown out. So the axons of the trigeminal[13] cells first grow out, then follow the axons of the facialis cells, then those of the glossopharyngeus and vagus.

The same was seen in the activation of the oculomotorius, abducens and hypoglossus nuclei which are activated by another influence of the same character.

[12] Bok. Die Entwicklung der Hirnnerven und ihrer Zentralen Bahnen. Dei Stimulogene Fibrillation. Folia Neurobiologica. Bd. 9, 1915. See also Bok, Stimulogeneous Fibrillation. The cause of the structure in the nervous system. Psych. en Neurologische Bladen, Amsterdam, 1915.

[13] Concerning the Trochlearis. See the first-named original.

Bok, considering the fact that the formation of the axis-cylinders in those cells took place under the influence of the current irradiating from the primary activating axis-cylinder, called this stimulogenous fibrillation, following the direction of that current in contrast to the outgrowth of the dendrites and
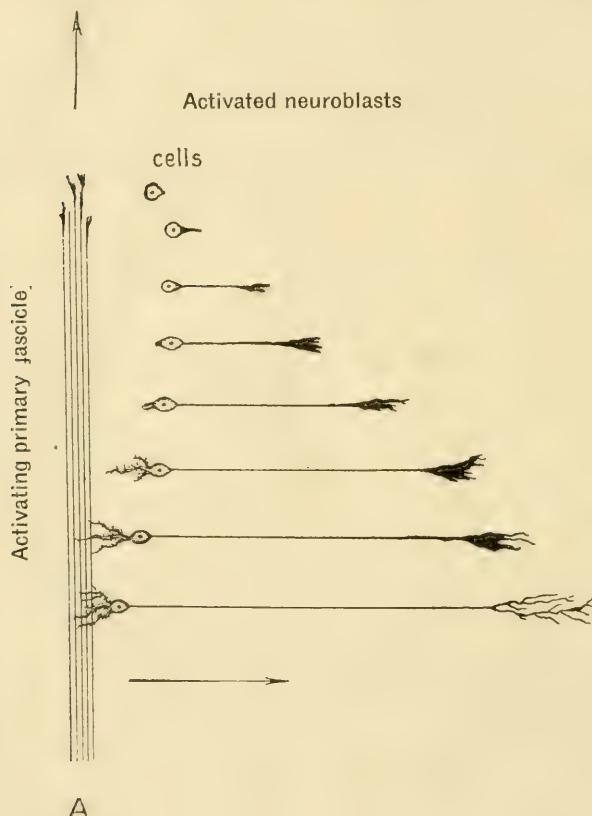
Fig. 4 The activation of adjacent neuroblasts by an amyelinated (growing) fascicle. The vertical arrow indicates the direction of growth of the activating bundle and the direction of its nerve current, which starts at A. The horizontal arrow indicates the course of the irradiating influence (current) perpendicularly to the activating bundle. Notice that the proximal cells are sooner activated (and have moved further) than the more distant ones. After Bok.

the shifting of the cell body,[14] both of which also only occur later and which move towards the center, i.e., against the current of the stimulus that proceeds to them.

This observation and Bok's interpretation of it are very important, and no doubt correct. It is evident, however, that the final end-point of the growing axis-cylinder can not be determined by this process alone, as was also realised by Bok, who came to the conclusion that the final connection was determined by the principal law of neurobiotaxis, viz., by the stimulative (simultaneous) correlation of the growing axis-cylinder and its end-point, i.e., the cell or dendrites with which it is going to be connected.

Bok thought that this could be effected by the fact that if two centers are in simultaneous stimulation the ideal line between the two is the path where the plasmodesms undergo the greatest influence of this relation. He called this the principle of 'doppelte Bahnung,' and thought that Einstein's (physical) law of attraction between synchronic energies also had some influence on it.

It seems to me, however, that the principle of 'doppelte Bahnung,' as laid down in this theory, can not explain from which of two simultaneously stimulated cells the axis-cylinders grow out, and that, even the adaptation of the protoplasm to the formation of the axis-cylinder, eventually a fibrillation of the neurodesms, then might begin in the middle between two cells which, as we know, it never does. Moreover, the expression "adaptation of protoplasm to its biological function" is too general an expression to explain anything.

It has appeared to me that the literature of recent years concerning the microchemistry of the neurones and the phenomena of tropism and taxis known and experimentally examined in other organisms, together with Bok's discovery, concerning the

[14] If the normal *stimulation* of the cell body is of little importance or eventually absent, the cell may also shift in the same direction in which the axon grows out. (See my paper on the autonomic nervous system. Journal of Physiology, 1908, vol. 37, p. 139.)

stimulogenous outgrowth of the axis-cylinders from the acti-
vated cell by and with the irradiating current from a primary or
activating axis-cylinder in its neighborhood, gives us a key of
exceptional importance to comprehend the phenomena of neuro-
biotaxes in general, and the contrasting behavior in outgrowth
direction between dendrites and axons and allows us to consider,
perhaps to explain, *how it is possible that a unit such as the neu-
rone is may exhibit two opposite directions of growth.*

EXPERIMENTS CONCERNING PHENOMENA OF TROPISM AND TAXIS IN PLANTS AND ANIMALS. KATAPHORETIC PHENOMENA

It is evident that, in any attempt to explain the neurobiotac-
tic phenomena, these must be compared with other phenomena
which are better adapted to experimental investigation. As
such we may mention the galvano-tropic phenomena in the growth
of plant-roots and the orientation of animals in the constant
current, about which we have obtained many data during the
last decennia.

As is known, the phenomenon of galvanotropy in plant-roots
was discovered in Hermann's laboratory by Müller-Hettlingen,[15]
who found that, if the sprouting seed of the bean (Vicia faba)
be exposed to a constant current, the tips of the root turn and
grow towards the negative pole (kathode).

An analogy[16] of this galvano-tropic phenomenon is found in
the galvano-tactic phenomenon described by Bancroft,[17] viz.,
that the tentacles and the manubrium of a medusa, Polyorchis,
during the transmission of a constant current turn towards the
kathode.

In the experiments with the latter this peculiar phenomenon was
observed, viz., that with a long-continued current the side turned
to the anode extended, becoming thinner and weaker; this last phenom-
enon being a symptom of decay, according to this author (vide infra).

[15] Müller-Hettlingen. Ueber galvanische Erscheinungen an Keimenden
Samen. Pflüger's Archiv, Bd. 31, 1883, p. 192.

[16] Not a homology, probably.

[17] Jour. Exp. Zoöl., vol. 1, 1904, p. 289.

As third example of galvano-taxis the phenomenon discovered by Verworn[18] in one-celled creatures must be mentioned here, viz., that these (ameba, for instance) on the transmission of a constant current through the surrounding medium, send out enlargements and finally shift in the direction of the kathode.

Verworn at first held the opinion that there were also Protozoa which shift under normal circumstances to the anode, and he therefore made a distinction between kathodic and anodic galvano-taxis. Later investigations revealed that the anodic galvano-taxis must be considered as being something different from the kathodic, and that the direction and shifting of the bodies observable in Protozoa under normal circumstances is invariably a *kathodic galvano-taxis*.

Also Boruttau (personal communication) holds the opinion that every real galvano-tropism is a kathodic stimulation phenomenon. This usual kathodic galvano-tropism can be brought into correlation with Pflüger's law, as has been pointed out by Loeb and Maxwell.[19]

As far as the anodic tropism is concerned, Loeb and Budgett[20] and after then Coehn and Barratt[21] found that when a protozoan, Paramecium, in pure water or in a weak solution of common

[18] Verworn. Die polare Erregung durch den galvanischen Strom. Pflüger's Archiv, Bd. 45, 1889. Verworn. Die polare Erregung der Protisten durch den galvanischen Strom (Fortsetzung). Pflüger's Archiv, Bd. 46, 1890. Verworn. Untersuchingen über die polare Erregung der lebendigen Substanz. 3te Mitteilung. Pflüger's Archiv, Bd. 62, 1896, S. 415. Verworn. Die polare Erregung der lebendigen Substanz durch den Constanten Strom. 4te Mitteilung. Pflüger's Archiv, Bd. 65, 1897.

[19] J. Loeb und S. S. Maxwell. Zur Theorie des Galvano-tropismus. Pflüger's Archiv, Bd. 63, 1896.

See also J. Loeb und Walter Gerry. Zur Theorie des Galvano-tropismus, II. Versuche an Wirbelthieren. Pflüger's Archiv, Bd. 65, 1897, S. 41. J. Loeb. Zur Theorie des Galvano-tropismus, III. Ueber die polare Erregung der Hartdrusen von Amblystoma durch den Constanten Strom. Pflüger's Archiv, Bd. 65, 1897, S. 308.

[20] Loeb und Budgett. Zur Theorie des Galvano-tropismus. IV. Mitteilung über die Ausscheidung electropositiver Ionen an der ausseren Anoden fläche protoplasmatischer Gebilde als Ursache der Abweichungen vom Pflüger'schen Erregungsgesetz. Pflüger's Archiv, Bd. 65, 1897, S. 532.

[21] Coehn und Barratt. Ueber Galvano-taxis von Standpunkt der physiologischen Chemie. Zeitsch. f. allgemeine Phyziologie, Bd. 5, 7, 1905.

salt, was influenced by a constant current, the movement was in the direction of the kathode, but that this direction of galvano-taxis may be reversed by placing the animal in a stronger (even physiological) solution of salt. If in the latter case the constant current was transmitted through it, a movement towards the anode was observable. This phenomenon of reversal, first observed in a galvano-tactic process, was confirmed shortly after in a galvano-tropic process, in the case of the root-tips of pease.

Gassner[22] found that an increase of salt in the medium influences the effect of the constant current in those objects also. When he increased the quantity of salt of the water in which pea-roots sprouted, the constant current could no longer cause a kathodic tropism. He was inclined to ascribe this to a diminution in the quantity of electricity running through the tip of the root, since the greater conductivity of the water (salt solution) caused a greater quantity of electricity running through the solution itself.

Schellenberg[23] obtained the same result, but went even farther, and on increasing still more the percentage of salt was able to obtain a reversed tropism, the root then growing to the *anode*.

If the percentage of KCl in the water was only 0.074 per cent the root-tip continued to grow kathodic galvano-tropic; if, however, the percentage was raised to 1 per cent, a distinct anodic direction in the growth appeared, and with a fair degree of exactness such a concentration of KCl could be found in which, after the transmission of the constant current, no tropism was evinced.[24]

[22] Gassner. Der galvano-tropismus der Wurzeln. Botanische Zeitung, 1906, Parts 9–11.

[23] Schellenberg. Untersuchungen über den Einfluss der Salze auf die Wachstumsrichtung der Wurzeln, zunächst an der Erbsen Wurzel. Flora, vol. 96, 1906, p. 474.

[24] It may be mentioned that the current strength which caused this tropism was but slight, and varied from 1/10 to 1/1000 milliampere, with a density of current of 0.0025 to 0.000025 milliampere per sq. cm.

That Elving's curves (which are also anodic) could be formed under these circumstances is out of the question, since Brunchorst found the current density necessary in this case to be about 0.2 milliampere.

Like Gassner, Schellenberg also seems to be inclined to ascribe the anodic tropism to the greater amount of electricity running through the water, or rather to the weakness of the current that runs through the root-tip and which should be too weak to cause a kathodic tropism, a supposition that seems to be accepted by Rothert,[25] though he admits that it has not been proved. If only a weaker current were sufficient to cause the anodo-tropic phenomenon, a smaller amount of electricity would have to do the same! These authors, moreover, do not explain biochemically why a weak current should cause an anodic tropism and a stronger current a kathodic one.

Coehn and Barratt (loc. cit.) tried to explain biochemically this *phenomenon of reversal of the galvano-tropism* in the following way. They assumed that on the boundary between the object used for the experiment and the surrounding medium (the water) a semipermeable membrane is present that possesses a different permeability for positive and negative ions. This assumption is quite legitimate, since the occurrence of such semipermeable membranes is a very common phenomenon in nature.

If we now assume that the permeability for negative[26] ions is greater in this membrane than that for positive ions, of the ionized NaCl or KCl (provided the concentration thereof in the surrounding fluid be greater than in the protoplasm) a larger quantity of negative ions will be transferred into the cell than of positive ions, and the cell will then be overcharged with negative ions and pass to the positive pole on the transmission of the constant current.

On the other hand, if the concentration of KCl or NaCl in the medium be less than in the cells, a larger quantity of negative ions than positive will leave the cell, and the cell-bodies, charged

[25] Rothert. Die neuen Untersuchungen über den Galvano-tropismus der Pflanzenwurzeln. Zeitschrift für allgemeine Physiologie, Bd. 7, 1907, p. 192.

[26] Such a special permeability for negative ions (anions) has been proved to exist in the case of blood corpuscles by Hamburger (Zeits. f. Biologie, Bd. 28, p. 405, 1891). Compare also Hamburger und Van Lier, Durchlässigkeit der rothen Blutkörperchen für die Anionen von Natriumsalzen. Arch. f. Anat. u. Physiol., Physiol. Abt., 1902, p. 492.

with a surplus of positive ions, will on the transmission of the constant current pass to the kathode.

If we accept this theory as correct, we shall have to assume that in ordinary circumstances—under which the kathodic tropism or taxis predominates—also a greater charge of positive ions is present in the cell-body of the ameba, or in the protoplasm of the tentacles or root-tips, than in the surrounding extra-protoplasmatic medium. This explanation is not generally accepted, but that the condition of the extra-protoplasmic medium is of great importance has also been emphasized by Loeb and Budgett, who are equally inclined to ascribe the exceptions to Pflüger's law (the anodic migrations) to alterations in the extra-protoplasmic medium. They refer to a phenomenon which may be exhibited by that side of an ameba or paramecium that is turned to the anode, viz., the extension of the protoplasm on that side, eventually followed by liquefaction. This anodic extension, first observed by Verworn (loc. cit.), is the first thing that appears when Protozoa are exposed to the constant current and precedes the real kathodic galvano-tropism.

Loeb and Budgett (loc. cit.) have submitted it to a more detailed examination and also came to the conclusion that this process is a result of the extra-protoplasmatic medium. Their explanation of this anodic phenomenon differs from the one given by Coehn and Barratt. They are, however, equally inclined to consider this phenomenon as due primarily to changes in the extra-protoplasmatic medium[27] in contrast to the phenomena of common tropism following Pflüger's laws of irritation. It may be mentioned still that the most favorable strength of current in those experiments with ameba was only 0.4 milliampere.

Besides these galvano-tactic and galvano-tropic phenomena of living protoplasm, we know of polar phenomena in dead organic substances rendered evident by the direction in which albumen shifts when subjected to a constant current: viz., the phenome-

[27] Perhaps this mode of explanation may be also applicable to the above-mentioned reversal of the galvano-tropism of root tips and to the anodal phenomenon observed by Bancroft (vide supra).

non of kataphoresis. I refer here to the investigations of Hardy,[28] which showed that as long as an albuminous solution is alkaline the particles suspended in it shift towards the anode on the transmission of a constant current, whereas they shift towards the kathode when the solution is made slightly acid.

One is apt to look for an explanation of this also in the fact that on the boundary between a colloid particle and the surrounding fluid, a double layer in the sense of the theory of Helmholtz-Quinke is present.

If now the solution is alkaline, a transmission of ions will take place, in consequence of which the albuminous particle itself becomes negative and thus shifts to the anode on the transmission of a constant current, while in the case of an acid reaction of the surrounding fluid the contrary takes place. From the reversibility of the kataphoretic phenomenon (Hamburger)[29] the curious fact thus follows, viz., that also proteid particles have the peculiarity that their electric character is determined by the reaction of the surrounding medium.

That here too, just as in the above tropism of the root-tips, an iso-electric condition occurs is clear.

APPLICATION OF THESE EXPERIMENTS TO THE GROWTH OF THE NEUROBLAST. THE FORMATION OF THE AXON

If, with these facts before us, we consider the phenomena which appear during the formation of an axis-cylinder[30] in an activated cell (which precedes the formation of dendrites—see

---

[28] Hardy. On the coagulation of proteid by electricity. Jour. of Physiol., vol. 24, p. 2881, 1899. Proc. Roy. Soc., vol. 68, p. 110, 1900.

[29] Hamburger. Osmotischer Druck und Ionenlehre. Wiesbaden, Bergmann, 1904, vol. 3, p. 68.

[30] It is hardly necessary to say that the fact that isolated ganglion cells, as in Harrison's experiments, may also send out axis-cylinders proves nothing against the following text. Harrison (loc. cit., p. 833) remarks that this is a process of self-differentiation entirely independent of external conditions. This is true to a certain extent, but we must assume that before it becomes a self-differentiation its differentiation has been induced to the neuroblast in former generations by external circumstances and that its doing this by itself is based on hereditary engrammatic qualities. It is better to see a problem in things than to explain them by a word which implies a still greater problem.

fig. 4), we shall first have to mention the fact that the stimulation center with respect to the surrounding tissue is negative, forming a kathode with reference to the non-stimulated surroundings, as physiological experiments abundantly prove.

Moreover the strength of the electrolytic potential differences occurring in the nervous system in consequence of stimulation appears to be of the same category as those that are applied in artificial phenomena of galvano-taxis (see above) since it may vary from 3 millivolt to 0.8 millivolt and lower, so that the forces developed here are certainly strong enough to influence processes of formative tropism and functional taxis.

Now, it may be the same whether this stimulated center is the body surface in or under which nerve cells lie or whether we start our deductions with a primary growing axis-cylinder which on its way passes neuroblasts. This negative potential not only runs along the primary axis-cylinder (fig. 4) but also, we may assume, as long as the axis-cylinder is not provided with an insulating medullary sheath, that this negative potential stands perpendicular to the length of the activating axis-cylinder (or body surface), irradiating from it.[31]

In accordance with this perpendicular irradiation of the electrolytic influence, or current, we see that th neuroblasts near the primary activat ng bundle send out ax's-cylinders perpendicular to the activating bundle, and that similarly perpendicular collaterals may grow out from the original (activating) axis-cylinders themselves.

In both cases, in the formation of collaterals as well as in the outgrowth of the axis-cylinder of the secondary (activated) neuroblasts, the axis-cylinder substance proceeds in the direction of the perpendicular irradiation of the stimulated fiber, i.e., to the anodic pole.

---

[31] The irradiative stimulus of naked axons is very clearly illustrated by the position of the dendrites of Purkinje's cells perpendicular upon the parallel fibers in the molecular layer of the cerebellum and of the dendrites of the motor cells on the longitudinal (naked) axons in the spinal cord of Petromyzon. See my paper, Ueber das Rindenproblem, etc., in the Folia Neurobiologica, Bd. 8, 1914, pp. 529–530

This first outgrowth which, in the beginning, can be complicated with an anodal katophoretic shifting of the cell body itself (fig. 4) may be entirely independent of a propagation of the nervous current itself along the newly formed short axis-cylinder. As soon, however, as this axis-cylinder is fit for nervous conduction its rate of outgrowth will be considerably increased, a much stronger negative current running in the direction of its growth to the anodal field.

Why does this anodic growth occur before the kathodic tropism of the dendrites and the cell body? I will consider this question in the light of the above-mentioned experiences.

We know that the neuroblast is embedded in an organic solution, the pericellular lymph, containing a good deal of potassium salts.

Macallum has emphasized that the amount of potassium salt external to the nerve cell is great and that a considerable condensation of this element is present on its exterior surface.

Now Verworn has shown that on the transmission of a constant current the first thing to appear is an anodal expansion of the cell body, thus showing that a change of tension may be localised, by electric influences, on the anodal pole.

We may expect that this extension, being under the influence of a considerable amount of K and Cl, derives certain chemical and tropic characteristics from it.

That this really occurs in nerve cells is proved by the chemical constituents of the axon, compared with those of the dendrites and cell body.

We know from the researches of Macdonald, Macallum, Alcock and Lynch that the axis-cylinder is distinguished from the dendrites and the cell body by a much larger quantity of potassium and chlorides[32] (which, according to Macdonald, may also contribute to its conductivity for the nervous current).

[32] MacDonald. The injury current of nerves, The key to its physical structure. · Report of Thomson-Yates Laboratory, vol. 4, 1902, p. 213.

MacDonald. The structure and function of nerve-fibers. Proceedings of the Royal Society, vol. 76, B. 1905, p. 322.

Macallum. On the distribution of potassium in animal and vegetable cells. Journal of Physiology, vol. 32, 1905.

Macallum. Die Methoden und Ergebnisse der Mikrochemie in der Biologis-

The large quantity of KCl then present around its colloidal substance will favor (according to the experiments of Gassner, Schellenberg, and others) the anodo-tropic character of the axis-cylinder.

The phenomenon of the formation of the axis-cylinder and its collaterals in the direction of the anodic field, may thus be so expressed that we say that the neuroblast embedded in a solution containing a good deal of potassium and of chloride exhibits, in harmony with the experiments of Loeb, Budgett, Coehn and Barratt, a tropism at the anodal side of the neuroblast and that the KCl constituents of the neuroblast gathering on this side thus increase (besides its conductivity) the anodo-tropic character of its colloidal substance. This anodo-tropic character of the colloidal substance of the axis-cylinder is, moreover, in harmony with Hardy's experiments on the kataphoresis of albuminoids.

Considering the fact, that the kataphoresis which genuine albumen and lecithin show is already generally an anodic one (Höber, loc. cit.) it is clear that the additional composition of the neurone and its surroundings still favors this, since the colloid particles of the young axon are embedded in a medium containing a quantity of KCl, that makes its preponderating reaction alkaline. Moreover the greater conductivity which KCl gives it, may cause the greater quantity of electricity to be led through it.

That the constituents of a peripheral nerve are strongly conveyed to the anode is also experimentally shown by Hermann, to whose experiments I return later (see p. 291).

From every standpoint indeed it seems that the conditions for the primary outgrowth of the axon along with the kathodic current to the anodic field have been realized in the nervous system.

chen Forschung. Ergebnisse der Physiologie von Asher und Spiro, Jahrg. VII, 1908.

Alcock and Lynch. On the relation between the physical, chemical and electrical properties of the nerves. Part IV: Potassium, chlorine and potassium chloride. Journal of Physiology, vol. 42, 1910.

Macallum. Surface tension and vital phenomena. University of Toronto Studies, No. 8, Physiological Series, 1912.

The outgrowth of the axis-cylinder begins in the chick embryo about the second day of incubation (Bok).

Not until much later—according to Cajal when the growth tip of the axis-cylinder has reached, or nearly reached, its end point (about the 6th day of incubation in the chick embryo, Bok)—does an outgrowth of the dendrites begin, which make their way in the direction of the stimulus, that is in the direction of the kathode.

THE FORMATION AND CONTRACTION OF DENDRITES. THE FINAL SHIFTING OF THE PERIKARYON

I believe that there is a principal difference biologically as well as biochemically between the anodic elongation of the axon and the kathodic tropism of the dendrites.

The primary growth of the axon is in the beginning not directed to a certain point, but merely from a certain kathodic center, the outgrowth of dendrites, however, is much more influenced also in the beginning by their final end-points.

Their tropism corresponds with the regular appearance of the law of stimulation of protoplasm and exhibits a kathodic character, probably related with a more advanced nervous function for which a further stage of development is necessary.

This kathodic growth direction, as well as the kathodic taxis, is the usual thing in nature and, as Loeb and Maxwell have shown, is in harmony with Pflüger's law. We only have to prove that there are no factors which might interfere with it and change it into an anodal elongation.

This question is the more important since it may be that in the first phase of outgrowth of dendrites, which is not yet accompanied by a secondary shortening of the dendrite and the shifting of the perikaryon, a kataphoretic process might introduce it or at least be involved in it. Anyhow, the kataphoretic qualities of the dendrites may never be such that they should counteract the kathodo-tropic process which certainly is the chief factor in the shortening (contraction) of the dendrite and the shifting of the cell.

Now we know from the chemical examinations of Grandry[33] and Macallum and Menten[34] that KCl is hardly present in the cell and the dendrite, so that for us there is no reason here to expect an anodal kataphoresis.

Looked at from the point of view of Hardy's investigations, which according to Greeley[35] we may apply to intra-protoplasmatic colloidal granules, there are perhaps more arguments which favor the shifting of the dendrites, and later of the cell-body, in the direction of the stimulus. For we know that the dendrites and the cell-body differ from the axis-cylinder by the presence of Nissl's substance which during life is probably in a more or less fluid condition (see Cowdry's papers[36] on the bio-chemical conditions of nerve cells). This substance is probably a derivative or compound of nucleic acid[37] and the presence of acid in it will, according to Hardy's investigations, promote the shifting of the colloids which are suspended in them to the kathode. While, therefore, the absence of a larger quantity of KCl does not prevent the first outgrowth of the dendrite from proceeding in the kathodic direction, the presence of acid nuclein derivatives would even promote it.

[33] Grandry. Recherches sur le structure du cylindre-axe et des cellules nerveuses. Bulletin de l'Academie de Bruxelles, 2 me Series, vol. 28, 1868, p. 304.
    Idem. Journal de l'Anatomie et de la Physiologie, vol. 6, 1869, p. 289.
[34] Macallum and Menten. Distribution of chlorides in nerve-cells and fibers, Proceedings of the Royal Society, vol. 76B, 1905, p. 217.
    Macallum. Die Ergebnisse und Methoden der Microchemie in der biologischen Forschung. Ergebnisse der Physiologie von Asher und Spiro, Jahrg. 7, 1908, p. 697.
[35] Greeley. Experiments on the physical structure of protoplasm of Paramecium and its relation to the reactions of the organism to thermal, chemical, and electrical stimuli. Biological Bulletin, vol. 7, 1904.
[36] Cowdry. The development of the cytoplasmatic constituents of the nerve-cells in the chick. Mitochondria and neurofibrils. Am. Jour. Anat., vol. 15, 1912.
    Cowdry. The relations of mitochondria and other protoplasmatic constituents in spinal ganglion cells of the pigeon. Internationale Monatschrift für Anatomie und Physiologie, Bd. 29, 1913.
    Cowdry. The general function and significance of mitochondria. Am. Jour. Anat., vol. 19, 1916.
[37] M. A. Van Herwerden. Ueber die Nuclear-wirkung auf tiersche Zellen. Ein Beitrag zur Chromidienfrage. Archiv f. Zellforschung, Bd. 10, 1913.

This substance perhaps also helps to explain the relatively late formation of the dendrites, since the nuclein substance does not appear until late in the cell body in the form of Nissl bodies which, originating from the chromatin of the nucleus (Scott),[38] pass through the nuclear membrane in a stage of development when the axis-cylinder has already completed its growth over a certain extent, and has even become fairly considerable.

Cajal[39] did not find this substance before the time when the dendrites start to grow out. Consequently when this substance is present in the protoplasm we observe a tendency in the protoplasm to shift in the direction of the stimulated field (the kathode) and to be followed by the contraction of dendrites and finally the shifting of the whole perikaryon in the direction of the stimulation field, i.e., in the direction of the negative electric field.

The difference of time between axis-cylinder outgrowth and dendrite formation thus would be a result of the general anodic kataphoretic character of genuine albumen and lecithin, the alkaline reaction of the pericellular lymph and the quantity of KCl salt present around the young cell and, further, the greater conductivity that this gives to the axis-cylinder on one hand, and on the other hand the late appearance of the nucleic acid derivatives in the protoplasm.

It cannot surprise us in this respect that only small dendrites are found on those cells whose chromatin is still entirely in the nucleus (granular cells), and that the smallest quantity of nuclear chromatin is found in those cells whose dendrites have developed most (motor cells, reticular cells and others).

Only the question would remain why the alkaline reaction of the body lymph, which is the same around the axis-cylinder and the dendrite, does not interfere with a kathodic outgrowth of the latter and this fact seems to prove the truth of the opinion

[38] Scott. On the structure, microchemistry, and development of nerve cells with special reference to nuclein compounds. Trans. Canadian Institute, vol. 6, part 142, p. 405, Dec., 1899.

[39] Cajal. Textura del sistema nerviosa del hombre y de los vertebrados, tomo 1, p. 528, Madrid, 1904.

of Loeb and Budgett that the kathodic tropism, following the law of irritation, is chiefly dependent on *intra*-cellular proto-plasmatic conditions and that the extra-cellular medium does not act such a part here as it does in anodal extensions.

Indeed, it seems more probable that the later outgrowth of the dendrites as well as their secondary contraction, including the shifting of the cell body is a process different in principle from the anodal outgrowth of the axis-cylinder, a process for which a greater functional completeness of the neurone is neces-sary, and that we may only say that the character of the chemical constitution of the dendrite is not such that it would interfere with it by a disturbing anodal process.

There are still three questions that may be mentioned in this discussion.

MONOAXONISM AND POLYDENDRITISM

The first question is why only one axis-cylinder leaves the cell, one which becomes complicated only by collaterals which proceed perpendicularly from it during its course, while from the cell-body, a large number of dendrites may and generally do grow out to several centers of stimulation (monoaxonism and polydendritism).

To explain the monoaxonism we may first consider what would happen if two kathodic currents traversed the young neuroblast at the same time. In a purely polar tropism, as galvano-tropism preëminently is, it is a familiar feature that the object under the influence of the current places itself so that the influence is equally great on both sides of the object. Only then does the state of equilibrium begin.

J. Loeb[40] in particular has shown this repeatedly, for example in his 7th Lecture, in which he speaks of radiating energy and heliotropism, and points out that the orientation of a simple object will continue until all its parts lie at the same angle with reference to the influence.

[40] J. Loeb. Vorlesungen über die Dynamic der Lebenserscheinungen. Leip-zig, Joh. Ambr. Barth, 1906, p. 171.

As long as the influence on right and left, or indeed on all sides, be unequal, the object will change its position until the state of equilibrium is arrived at and the influence is the same everywhere.

Let us now apply this to a charged field from which a stimulus irradiates and passes to a cell in the neighborhood.

It will be clear, without anything further, that the outgrowth of the axis-cylinder in the current from the kathodic field to the anode has a state of equilibrium only in the course, that is, lengthways, of the current, i.e., in a collateral growing out perpendicularly or, where the growth stimulus proceeding from the irradiating current activated a cell in its neighborhood, the latter must send out its axis-cylinder also perpendicularly from the source along with the current. This explains the peculiar fact of collaterals of axis-cylinders in the commencement of their course having invariably a strictly perpendicular position with regard to the axis-cylinder.[41]

The irradiation current which radiates sideways from an activating axis-cylinder must naturally move in a direction perpendicular to this axis-cylinder. This is a physical fact that is only changed at the growing point of the activating axone.

It will thus be seen that the presence of one axon, as well as the perpendicular position of the collaterals on the axis-cylinder, are but natural consequences of the perfect bipolar character of the current. Now the same holds good if two or more differently running tracts, or differently placed centers, activate one cell simultaneously. We then may also expect only one axis-cylinder in the resultant line of the two current directions (two bio-electric fields), since only in this line the equal influence on both sides of the growing point, the energetic equilibrium, is realized.

What will be the case if two or more activating centers are present not acting simultaneously? One of these activating centers has to be the first and causes the initial outgrowth.

[41] At the time when coloration and impregnation methods were not so advanced as now, the differential diagnosis of collaterals and dendrites was sometimes made on account of the perpendicular position of the latter on the axon.

If, however, an axis-cylinder has started to grow, we may expect that the favorable conditions which it offers for the current, on account of its greater conductivity, are such that the obstacle to the formation of a new axon at some other place is so much greater, that the current will take the present path of enlarged conductibility, the course of which it may influence perhaps without, however, causing a new axon to grow out, the point of application of forces being localized.

The conditions with the dendrites are quite different.

This process is by no means necessarily limited to one part of the surface of the cell since its whole body containing Nissl substance is equally sensitive and any stimulation may cause protoplasmatic shiftings in their direction, whereby the principal dendrite and finally the shifting of the cell-body itself will doubtless take place in the direction of the maximal stimulus.

In other words, if another stimulus than the one which formed the axis-cylinder reaches the cell, it will form no new way out, since this would require more energy than a following of the present path of greatest conductivity, but a new stimulus coming from another center, may produce—or even must produce— a new dendrite. Since the perikaryon is equally sensitive (except the axon hillock) to it everywhere and since already existing dendrites are not in its path, the nearest cellular or dendritic surface will be the point of application for its influence, i.e., for the formation of a new dendritic outgrowth.

THE SELECTIVITY IN THE PROCESS OF NEUROBIOTAXIS IN HARMONY WITH PSYCHOLOGICAL LAWS

I now come to the second and most important point in the tract formation, that which determines the selectivity of the definite connections.

It has escaped the observation of all the earlier investigators that the selectivity of the tract formation depends upon simultaneous, or better, correlative, stimulation. Cajal assumed chemical secretions coinciding with stages of evolution, also ascribing an influence to the glia cells in the secretion of such "substances attractives" and *without pointing out by which factors*

these stages of evolution were defined, which he could not do since his conclusions were chiefly, if not solely, based on ontogenetic, that is engrammatic observations. Held speaks of a "Prinzip der Auswahl," upon the character of which he does not enter, and with regard to his own researches Harrison[42] justly remarks:

> There is nothing in the present work which throws any light upon the process by which the final connection between the nerve and its end-organ is established.
> That it must be a sort of specific reaction between each kind of nerve fiber and the particular structure to be innervated seems clear.

That the relationship for the final connection, which holds good in the central nervous system for the dendrites and the cell-shifting as well as for the axis-cylinders exists in the correlative, mostly synchronous stimulation condition of the elements, I first deduced from the selective character of the cell shifting, and this could be further clearly demonstrated by the axonic connections existing in the nervous system. It even explains a series of peculiarities in the course of the fiber tracts which otherwise confronted us as constant but inexplicable facts, especially in the so-called central motor tracts such as the pyramids.

This fundamental law of neurobiotaxis shows us not merely that the fundamental law of association in psychology is at the same time an anatomical law, but also how wonderfully polar the whole character of tract formation is, an how it therefore falls within the range of the galvano-tactic and galvano-tropic phenomena.

In order to explain this phenomenon of selectivity in an electro-chemical way, I must draw attention to the following points.

It is presumed that the presence of potassium salts has the peculiarity that it greatly increases the conductivity of the axis-cylinder for the electrolytic current.

There is even an inclination to ascribe the strong conductivity of the axis-cylinder, as compared with the synapse, to the high percentage of potassium salts in the axon (MacDonald, Macallum).

[42] Harrison. The outgrowth of the nerve fiber as a mode of protoplasmic movement. Jour. Exp. Zoöl., vol. 9, 1910, p. 787.

We may assume that a state of stimulation once raised at the beginning of that axis-cylinder will proceed rapidly—it is even supposed under a gradually increasing force (the axis-cylinder increases in caliber centrifugally: Johnston,[43] Tretjakoff[44])—and a current of relatively great negative electric potential reaches the growing point of the axis-cylinder.

If we now assume that in the neighborhood of this growing point two nerve cells lie, one of which is already in a condition of stimulation but the other not, on which of these two cells will this growing point then exercise the greatest influence, and which cell will exercise the greatest influence on the growing point?

As we know, the cell which has just been stimulated will be in a state of greater electrolytic dissociation than the cell which is in rest.

The negative ion current which runs along the axis-cylinder in its neighborhood, will find its natural selection in this strongly dissociated field, and not in a cell which is not stimulated and, being relatively indifferent with respect to this growing axis-cylinder, does not form a place of selectivity amid all the other passive (non-stimulated) cells which, so to speak are corpora aliena for it.

Now we know (see above) that the dendrites of a cell begin to grow out about the time when the telodendria of an axis-cylinder reach it or approach very near to it, and this is in striking agreement with the explanation given here of the neurobiotactic processes, because at the moment when the approaching and stimulated axis-cylinder comes into the vicinity of the cell, the influence of the approaching kathodic potential difference will make itself more strongly felt, and a shifting of the protoplasm into its direction, i.e., a tropism towards the telodendria, is induced, which is a kathodic phenomenon of irritation like most tropisms under normal circumstances where no special conditions for a reversal occur.

[43] Johnston, J. B. Additional notes on the cranial nerves of Petromyzon. Jour. Comp. Neur., vol. 18, 1908.

[44] Tretjakoff. Das Nervensystem von Ammocoetes. I. Das Rückenmark. Archiv f. mikr. Anat. u. Entwick., Bd. 73, 1909, plate 24, fig. 11.

A closer approach of the two neurones, a contiguity, will be the result. That this will not (or not easily) occur if the growing axis-cylinder reaches a cell in rest, may result from the fact that this passive cell, or neurone, is not in that strongly electrolytically dissociated condition and possesses no considerable electrical potential difference from the surroundings. Or to put it otherwise, the passive, non-stimulated cell has thus no other significance for the growing axis-cylinder in its vicinity but that of a corpus alienum, i.e., it is fairly indifferent to it.

As far as concerns the fact that axonic endings never communicate with axonic endings and dendritic endings never with dendritic endings, no further explanation is necessary from the standpoint of polar electrolytic conditions accepted here, which necessarily implies that homonymic outgrowths do not act on each other.

FASCICULATION OF AXONS. IMPROVEMENT OF THE NERVOUS PATH

One might ask in this connection why nerve fibers, if homonymic forces repel each other, tend to group together in fiber-tracts or bundles, as we always see even if they do not end on the same level (as the pyramidal tracts).

This process may, however, be analogous with the mono-axonism, that an axon shall tend to place itself in the way of the current, and if now such a current reaches a pluricellular center it is not strange that the chief resultant line for the outgrowth of one cell is also the state of equilibrium for the outgrowth of the other adjacent cells. The orientation of a number of nerve-fibers (axons) from a cell group into one bundle[45] may be no more than a repetition of the same process concerning the neurofibrils in one axon, which tend to a state of central equilibrium in the axis of the neurite.

Perhaps also a sort of magnetic field formed by equally running currents may exercise an attraction here. Such a magnetic field is also present around colloidal threads.

---

[45] Perhaps also a sort of magnetic field formed by currents running parallel exercises an attraction here.

Just as we saw that with the dendrites of one cell the question is different (see above), we also see that dendritic outgrowths of more cells rarely fasciculate in a bundle. The latter would be only the case if only one stimulation center attracted them all, which rarely happens.

As far as concerns the neurites, I will discuss below still another point in which it seems to be indicated that conditions which hold good for one neurite may also hold good for a collection of neurites, for a bundle.

I will not leave, however, the question o˙ interneuronal connection without emphasizing that the greater conductivity of the axis-cylinder (based on much more K and Cl) in comparison with the dendrites, gives a peculiar character to the shifting of the nerve cell in the direction of the center of stimulation. This shifting causes a shortening of the dendritic path and a lengthening of the axonic path for the nervous current and consequently a diminution of the resistance, or if this expression be less happy, an improvement in the conductivity.

It seems probable that the retardation which the nerve current experiences in the synapse is diminished by this process. Very interesting in this connection is Mauthner's cell in fishes, where the transmission of the afferent current takes place in part on the axon cap itself (Bartelmez[46]), and where probably the least resistant synapse is formed.

Similar facilitation of the transmission of the current may be seen in other structures concerned with equilibrium, e.g., in the basket cells of the cerebellar cortex where, as Oudendal,[47] among others, has shown, fibrillae of the basket are continuous with the fibrillae in the bodies of Purkinje's cells.

Since the shortening contraction of the dendrites in such cases as the descent of the facialis nucleus in mammals is accompanied by a lengthening (extension) of the axis-cylinder (fig. 5), we may ask whether there is not an analogy of this process

[46] Bartelmez, G. W. Mauthner's cell and the nucleus motorius tegmenti. Jour. Comp. Neur., vol. 25, pp. 87–128, 1915.

[47] Oudendal. Ueber den Zusammenhang der Auslaufer der Korbzellen mit den Zellen von Purkinje in der Rinde des Kleinhirns. Psychiatrische en Neurologische Bladen, Amsterdam, 1912.

with the process seen in muscles, which at the closure of the
current exhibit, besides the contract on at the kathode, also
an extension at the anodal pole, the broad analogy between the
law of stimulation for muscles and nerves being known.
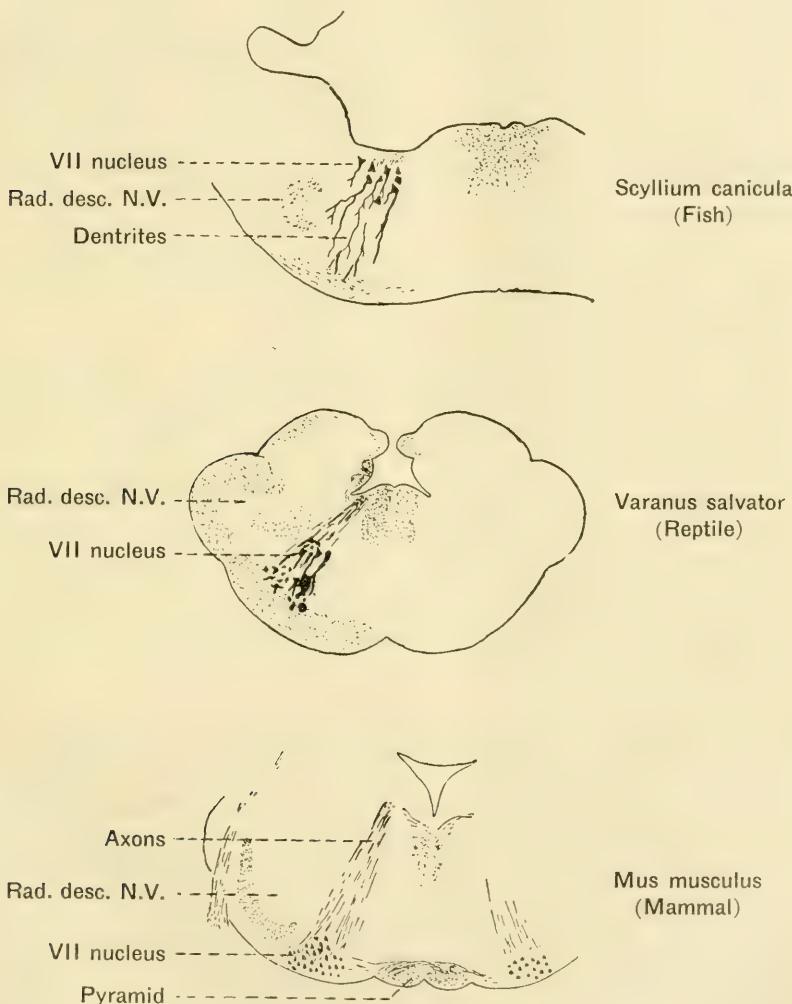
Fig. 5 Migrations of the motor facialis nucleus in the animal series, which
is correlated with a shortening of the dendrites and an elongation of the axis-
cylinders.

THE FORMATION OF THE MEDULLARY SHEATH

The third point that might be mentioned in this discussion is the question as to why most axis-cylinders in the central nervous system get a medullary sheath, and why this medullary sheath is not present on the cell body and the dendrites.[48]

If one were content here with a teleological explanation, it would be sufficient to say that the presence of a myelin sheath around the axis-cylinder probably has the function of insulating the current, and that an insulating sheath should not occur in places where this current proceeds from one neuron to another (dendrites, cell body, telodendria). And yet that would not bring us one step nearer to the solution of the question as to the way in which the process of myelin accumulation is effected by the axis-cylinder.

Let us endeavor here also to trace the influence which may lead to the accumulation of myelin around the axon, and why it is not accumulated sheath-like or otherwise in the cell and the dendrite.

That the primitive axis-cylinder itself is able to form myelin is proved most clearly in the central nervous system, where the cells of Ranvier (i.e., of the neurilemma) which may have to do with it in the peripheral nervous system, do not occur, and other adjacent (glia) cells are but seldom found provided with myelin granules.[49]

---

[48] I do not refer here to the medullary sheath around the peripheral fiber of a sensory root, which is a dendrite anatomically and ontogenetically (it develops later than the central process). In the millions of neurons in the nervous system this is the only exception, which certainly requires explanation but at present need not disturb our reasoning concerning the central pathways. The peripheral nerve fibers—especially the sensory ones—do not seem to be the most adequate material to elucidate the questions involved here, since they seem to require more explanation instead of helping to elucidate these questions. Moreover the fact that spinal ganglion cells belonging to the sensory system of the skin receive stimuli from other neurones (of the sympathetic system—Dogiel) proves that nervous currents may also run toward their periphery.

[49] Vignal. Le développement des élements du systèm nerveux cérébro-spinal. Masson, Paris, 1889. See also, Ariëns Kappers. Recherches sur le développement des gaines dans le tube nerveux. Petrus Camper, Amsterdam, vol. 2, part 2, 1902.

We know, from the researches of Ambronn and Held[50] that myelin formation is greatly affected by the function of the tracts, and consequently strongly influenced by the stimuli passing through it.

I have already referred to the fact that the genuine albuminous substance and also the lecithin which forms the chief component of the myelin sheath generally exhibit, under normal circumstances, an anodic kataphoresis.

Concerning the myelin itself this has been experimentally shown by Hermann, who described its connection to the anode as "eine der gewaltigsten microscopischen Erscheinungen," he ever witnessed.

Putting a part of a peripheral nerve of a frog in a constant current in the line connecting the electrodes (which, however, remained at a distance from its ends), he saw a vigorous outflow of the nerve content—especially the myelin—at the anodal pole of the nerve, where it collected in a mass.

Reversing the current, this myelin could again be absorbed by the nerve and the myelin flowed out at the other (then, anodic) end.

The tendency of the peripheral nerve constituents—chiefly its myelin—to move in the direction of the anode is clearly proved by this experiment.[51]

If now we apply this phenomenon to the structure of the axon in the central nervous system we may expect that the nerve current which has—as pointed out above—an anodal direction, will convey the lipoid substance, even that which is produced by the cell itself, chiefly in the axis-cylinder; but, since from this axis-cylinder an irradiation current of the same character flows out, the myelin is necessarily conveyed to the periphery of the nerve fiber.

The difficulty consequently is not why only axis-cylinders have myelin and why this myelin is conveyed from the center

[50] Ambronn und Held. Ueber Entwicklung und Bedeutung des Nervenmarks. Sitzungsverichte der Kön. Sächsischen Gesellschaft der Wissenschaften, 1895.

[51] I am much indebted to Prof. Höber (Kiel) for calling my attention to Hermann's paper, which was unknown to me when I started to write this article. It is found in Pflüger's Archiv, Bd. 67, 1897, p. 240.

to the periphery and there gathering sheath-like round it;
but the greater difficulty is why it remains there, and why is it
not conveyed further away from the sheath.   Perhaps in the
beginning of sheath formation this really occurs (some glia
cells and lymphocytes are found richly provided with myelin-
like or fatty granules), but when its formation becomes more
abundant it prevents by its nonconducting character the anodal
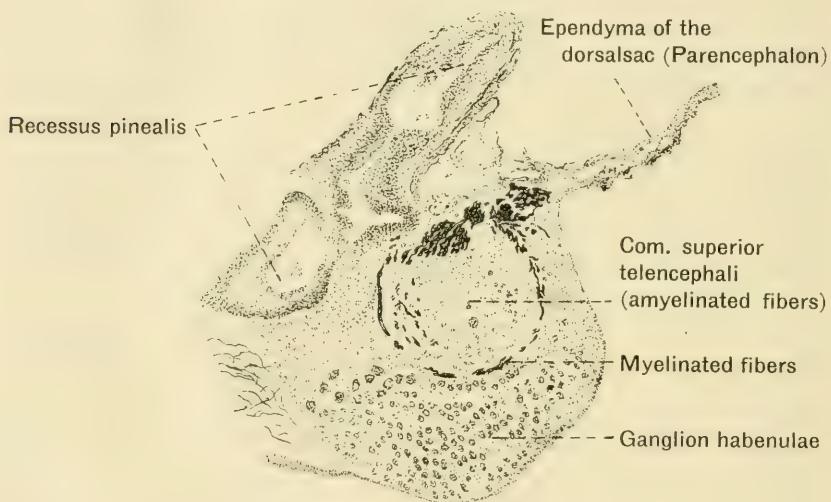
Fig. 6   Sagittal section of the habenular ganglion of Scyllium canicula, show-
ing the position of unmyelinated fibers surrounding the myelinated fibers.

current from extending its course and consequently its conveying
influence (kataphoresis) beyond the wall of myelin which thus
thickens more and more.

An induced anodic condition of the direct periphery might
then also cause lecithin substance of surrounding tissue (Ran-
vier cells) to gather on the sheath.

Why we do not find an accumulation of the same substance
at the apex of the axis-cylinder, why the telodendria remain
free from it, is difficult to explain.   Perhaps that the conveying
character of the current for this substance is so considerable
there that it does not remain there when formed.

In connection with the accumulation of myelin in the periphery of the axis-cylinder I wish to mention a fact which struck me repeatedly in the study of the cerebral commissures of ower animals, where (e.g., in the commissura superior habenulae of plagiostomes fig. 6) we frequently observe that the medullated fibers are arranged in the decussating bundle on the periphery of the non-medullated fibers. The same fact struck me often in the fasciculus retroflexus, especially in Arius.

Sheldon,[52] too, noticed this in his study of the olfactory tracts and centers in teleosts, and he makes the same remark with regard to some thalamic tracts. Whether this is to be explained as a repetition of the same process—an analogy—of peripheral accumulation of myelin in the medullary sheath, I do not venture to say. It seems probable, since we saw that also in another respect (monoaxonism and fasciculation, see above) the principle that holds good for an axon seems to hold good also for a collection of axons.

Here, however, we transgress the limits of a scientific hypothesis, which, though not pretending to be more than a mere hypothesis, must be founded on facts.

I would be perfectly content if this short note might stimulate others to think about these matters. The dynamic polarization of the neurone and its biologic character still require a good deal more light than has as yet been shed upon it, and is worth the attention of our best physiologists and biochemists.[53]

RÉSUMÉ AND CONCLUSION

From the shiftings exhibited (phylogenetically) by the cells of the motor nuclei it appears that those parts of the neurone that receive the stimuli (dendrites and cellular body) are formed and directed to those stimuli trying to approach their center.

[52] Sheldon, R. E. The olfactory tracts and centers in teleosts. Jour. Comp. Neur., vol. 22, pp. 177–339, 1912.

[53] I have only one more remark to make. Darwin once said that plants think with their roots. He did not mean this in a literal sense, of course, but that there may be some similarities between the sensibility to certain stimuli and the behavior of the roots of plants (or other centers of growth) and parts of the nervous system, chiefly the axons, does not seem so very improbable.

Further researches show that this influence is found only on such nerve cells as have already a certain previous indirect affinity with those impressions, or with the region where those impressions accumulate, and it can be proved that this affinity consists in a simultaneous or successive condition of action (stimulative correlation); and that consequently, in the material arrangement in our brains, the law appears which has been long since acknowledged to be one of the main laws for the development of our mental capacities, viz., the law of association.

The acknowledgment of correlated function as the fundamental factor in the arrangement of the cells and dendrites induced me to investigate whether the same law could be shown in the final course and connection of the axons to lower or higher centers (so-called central-motor paths and higher sensory neurones), and a careful comparison of the regions where such paths begin and terminate, showed, that here too such an associative affinity could be pointed out, that this affinity determines the place where the axon will end, and explains a number of peculiarities in the ending of such paths, e.g., throws a light on the singular fact that the pyramidal tracts do not originally terminate in motor, but in sensory regions.

Under this fundamental law, that *neurobiotactic processes occur between correlated systems*, the tropism of the dendrites and cell body takes place in an opposite direction to the nerve current, i.e., towards the center of stimulation: *stimulo-petal*, whereas the course of the axon conducting the impression farther is in the same direction as that current: stimulo-fugal or (more correctly) *stimulo-concurrent*.

That, however, also the development of the axon is a consequence of the stimulus has been proved by Bok, who in an equally convincing and ingenious way showed that at first the axon does not conduct a stimulus irradiating in the nervous system, but that on the contrary, this stimulus forms the axon so that also here a stimulogenous formation occurs, described by Bok in a very important contribution to our knowledge of neurobiotactic processes, under the name of stimulogenous fibrillation.

Taken all in all, we can say that the stimuli which arrive in the nervous system, especially the relation between those stimuli, mold the material substratum of the mind; this correlation is the primary force, and expresses itself in the material arrangements of our nervous system.

This correlation of stimuli thus plays the fundamental part, in all processes of neurobiotaxis, in which, however, the dendrites and the cell body grow towards the stimulus center stimulo-petal, whereas the axon grows away from the stimulus center, with the influence irradiating from it: stimulo-concurrent.

The question is now: how can we explain these different tropisms in the nervous system; how can it be, that one nerve unit, the neurone, shows such a clearly *opposite polar difference*, that one part of its protoplasm approaches the source of stimulation (*stimulo-petal dendrites and cell body*), while the other grows with the direction of the stimulus-irradiation proceeding from it (*stimulo-concurrent axons*)?

In order to find the solution of this problem, we may study the other tropisms in nature, which are more accessible to experimental research, especially the galvano-tropisms.

In galvano-tropisms we find phenomena which remind us most forcibly of the manifestations in the nervous system just described.

By *galvanotaxis* we understand the fact that a living being or part of it, when placed in a constant electric current of certain strength, is inclined to turn towards a certain pole, in most, or in nearly all cases towards the electro-negative pole (the kathode). Thus the root-tips of plants grow towards the electro-negative pole, monocellular animal organisms move in that direction.

The process is however reversible. By putting the object, such as the root-tips of growing plants or the monocellular animals in a stronger solution of chloride of potassium or sodium (which at the same time increases the conductivity of the solution) the tropism is reversed and goes towards the positive pole (anode).

Albumen also shows a shifting in a galvanic current (*kataphoresis*).

Contrary to the above-mentioned tropism, the shifting of

albumen and lecithine takes place under ordinary circumstances (that is to say in the circumstances in which it usually occurs in animal bodies) generally towards the positive pole. Addition of potassium also enhances the anodic character of this process, and the substance of the axon and myelin sheath of a nerve root, just cut from the body, shows in a galvanic current even a very strong displacement toward the positive pole (Hermann).

By acids the removal of the albuminous substance *may be reversed*, however, and directed towards the negative pole.

There is much evidence that these galvano-tropic and kata-phoretic experiments are applicable to the formation of the nervous system by the stimuli that reach it and act in it.

We know from the *negative variation* that a part of our nervous system which is stimulated forms a negative pole, a kathode, with respect to its surroundings, which in other words form an anodic field with regard to the center of stimulation.

The nerve-cells which are found in the surroundings of this electro-negative center of stimulation, will first show an anodic offshoot going in the same direction as the radiation from that center of stimulation, on account of the anodotropic character of their protoplasm. This anodic extension, will derive chemical and tropic characteristics of the potassium and chlorides in which it is imbedded.[54] In consequence a larger quantity of potassium chloride is found in the axis-cylinder than elsewhere in the neurone (as Macdonald and Macallum and Menten showed independently of each other and in different ways). This large quantity of chloride of potassium (conformably to the above-mentioned experiments with root-tips and ameba) will again enhance the *anodotropic, in casu stimulo-concurrent* char-acter of the axon, and besides it increases its conductivity.

Not until much later do the dendrites appear, and somewhat later still the cell body begins to move in the direction of the stimulated electro-negative center.

[54] Why so much Cl is found in the axonic part of the neurone is unknown. It seems possible to me that this is due to the anodotropic character of Cl, this being an anion. A greater permeability for anions might then be the cause of the enhanced anodotropic character of the colloid substance of the axon. There is much in favor of this, that it would be rather the chlorine than the potassium.

This *stimulo-petal, kathodic tropism of the dendrites and of the perinuclear protoplasm* is probably a more complicated phenomenon, which however is not counteracted by K and Cl, since this does not occur to any considerable amount in those parts. On the other hand, it may be favored by a kathodic kataphoresis since it coincides with the appearance of the nuclear acid derivatives, known as Nissl's bodies, and does not take place until the axon has nearly reached its terminus and the neurone is therefore in a much greater state of perfection (Cajal). This kathodic tropism, followed by a gradual shortening of the dendrite and a displacing of the cell itself (as in most kathodic tropisms), is in accordance with the phenomena of kathodic stimulation, according to Pflüger's law (Loeb and Maxwell, Boruttau), as these become apparent in animal protoplasm susceptible to stimulation (e.g., also in ameba under normal circumstances) and causes these parts of the neurone to find their way to the electro-negative field which is in a state of stimulation.

It is probably accompanied by a facilitation of a stimulus-transition at that place at the moment when the galvanic current which appears in the nervous system makes itself felt (*the enhanced sensitiveness at the kathode* well known in neurology).

Thus we find in the first development of stimulo-concurrent axons a consequence of the enhanced anodotropic character, experimentally proved, of their substance, strengthened by the large quantity of K and Cl, while the formation and contraction which takes place much later of the dendrites, and the displacement of the perinuclear protoplasm to the kathode is a special case of Pflüger's laws, not counteracted by any amount of KCl, perhaps even favored by nuclear acid-derivatives.

Such may be the explanation of the dynamic polarization of the neurone. It does not, however, tell us anything about the final connection of the axis-clyinder.

This final connection is always a territory or cell which has a correlated activity, that is a simultaneous electrolytic dissociation with it. Non-stimulated centers are all equally indifferent to it, i.e., corpora aliena to it. We further saw that *monoaxonism* is a result of the *effect on the same pole* (a resultant line of different

forces on the same point of application), while *polydendritism* is possible and even usual on account of the fact that their formation is *not* a resultant line of different forces *on the same pole* (mathematically expressed of different forces on the same point) of the cell body since the perinuclear and dendritic protoplasm is equally sensitive everywhere to the kathodic influence and may respond at several different places to several stimuli of different origin, each of which may affect it on that part of its *ubiquitous receptive surface* that is nearest by.

The ability of the neurone to receive at the same time more than one stimulus by different dendrites and to lead their compound along one axis-cylinder, may be considered as the material expression of the formation of a compound impression from different perceptions, and this compound again acts as a factor in the formation of higher, more complicated compounds, if the axis-cylinder runs in the cerebral direction. If it runs in the aboral direction, the axis-cylinder is the final common path leading to a somatic effector center.

It seems hardly necessary to emphasize that I do not believe that *nervous life* and still less its psychic, conscious realization are, or even could be, explained by such considerations—provided they are right. They may, at the best, give us an idea of some physico-chemic processes that accompany its evolution and explain the form in which our nervous elements appear. After all, "life" and its "données immediates" (Bergson)[55] remain as self-imposing truths, that are revealed in but not explained by any phenomenon whatever.

[55] Bergson. l'Evolution Creatrice. Felix Alcan. Paris, 1907.

FURTHER VERIFICATION OF FUNCTIONAL SIZE CHANGES IN NERVE CELL BODIES BY THE USE OF THE POLAR PLANIMETER

DAVID H. DOLLEY

From the Pathological Laboratory of the University of Missouri

THREE FIGURES

INTRODUCTION

Kocher ('16) has recently published several papers which make a sweeping denial of any morphological evidence of functional activity in the nerve cell body. He bases this conclusion chiefly on his failure to find constant differences in the average size of cells between exercised and undisturbed animals by the use of the polar planimeter. It became necessary, therefore, for the writer to test his own positive finding of functional changes by this method.

The writer has, indeed, used the polar planimeter extensively to determine the areas of constituent sections of individual cells in one micron series. This gave part of the data necessary in calculating individual cell volumes by the prismoid formulas. Some of these results are published ('14), some are not. It did not appear then, on objective as well as theoretical grounds, that the method by itself would add any essential information regarding the functional reaction that was not already supplied from average of diameter measurements. This the present results confirm.

It is further my more personal task to reply because the criticism of Kocher is directed entirely at myself, and patently aims to discredit methods, technic, and conclusions. One only finds advantage in a censoriousness which overshoots the mark, but I deprecate being singled out from the large company of workers who have been convinced that there are morphological evidences

299

of activity. Among these there is noteworthy unanimity when the phases with which they worked and the parts which they studied (differentiation) are taken into account, as I have attempted elsewhere to show ('11 b). Indeed, from the literature with which I am familiar, the score stands at present as eighteen who are willing to admit changes of one sort or another against two who are skeptical (Eve and Kocher).

Particularly, the credit must go to Hodge, the pioneer. It has been the writer's fortune to do no more than confirm every finding of importance which Hodge made. Furthermore, without these findings of Hodge and his deductions therefrom, and without the contributions of his immediate successors toward filling in the gaps, the interpretation of the sequence' of events in the more highly differentiated cells would have been enormously difficult for a single investigator, if not impossible, even though he profited as much as one could by the advance of cytology in two decades and more. And cytology is just what is meant.

AN ANALYSIS OF THE DENIAL OF A FUNCTIONAL MORPHOLOGY

Were it not for the technical difference of method, repetition of Kocher's work would not be necessary, for a critical analysis of his paper will easily show that certain of his essential conclusions do not mean what he appears to think they do, but on the contrary afford a confirmation of an essential principle of nerve cell function. This critical discussion will be taken up first, as it explains why there is no need for further experimental data than is submitted.

The writer has divided the progress of functional activity from rest to organic exhaustion into thirteen stages for the Purkinje cell. Kocher states: "Representatives of practically all these types of cells were found in my specimens, from the resting control animal, as well as from those animals exercised for one, two and a half, and five hours." These stages were so definite that he counted over three thousand cells in order to determine the varying distribution (Kocher, table 3).

Our objective findings therefore are the same; the stages exist; there is no rigid morphology of the cell. He does not explain

them; he does not appear to think they need explanation; he does not even consider why he finds them with his technic when he is so harsh with me about my technic; but he sees them. Coming as this confirmation does from a professed critic, with the prestige of a great laboratory behind it, it will doubtless carry unhoped for weight. The confirmation imposes the greater debt in that this arbitrary division of a continuous process was carried to a degree which on its face must have appeared suspicious, though the number was due to the coördinate inclusion of intermediate stages, and the division was a practical one for study.

The effect of varying function between one animal and another must be either qualitative or quantitative, granting that there is an effect. For the purpose of further analysis of Kocher's findings and conclusions, these possibilities must be considered separately, and the question of qualitative differences will be taken first.

His final conclusion which relates to this point is: "Furthermore, no qualitative differences in histological characters could be found between fatigue and resting nerve cells." Or, as it reads somewhat differently in the text: "There are neither progressive changes in the morphology of the cells from rest to exhaustion, nor are there any qualitative or quantitative differences in type of cells from resting and fatigued or even exhausted *animals* (italics mine). Qualitative cellular difference between animals in relative degrees of activity is what he wishes to specify, and assuredly there is none, if representatives of the thirteen stages, in orderly relation, are to be found in all, and only those. But Kocher is artlessly misled because he finds all stages in the 'control' as well as the exercised animal. So his conclusion of lack of qualitative difference does not mean what he thinks it does, that nothing has happened. On the contrary, it is a fundamental conclusion that qualitative differences from function are to be ruled out. Instead of being destructive to me, this is the first induction I should wish to be confirmed, since it throws comparative function on the quantitative principle. It is only that our opinions of the significance of an identical conclusion differ.

It is for qualitative changes for which the main search has been made, and it is on this point that many interpretations have foundered. Kocher, so far as he expected qualitative differences, predicated it on the idea that the cells of an animal pursuing its ordinary course are static. That, though possibly not exactly accessory appendages, still they are unaffected by the to and fro swing of ordinary existence. He simply neglects the conception which came in with cellular biology that every phenomenon of life of the organism is referable to its cells. For he speaks of the "resting cells of the controls" as if all cells in the undisturbed animal are necessarily static. Only when the extraordinary thing happens then, like being chased around in a treadmill, or overdrugged, or cut for appendicitis, should changes be expected, and these of a peculiar, not to say specific nature, to fit the assault on the integrity of the cell? When they do not appear, it is necessary for him to believe that nothing has happened. But the most ordinary vital phenomenon is a cellular phenomenon just as well, and must be correlated with the whole range of extraordinary phenomena. Were nervous phenomena qualitative, an infinite range and variety would be necessitated.

No animals can be conceived to be static, in one fixed state. Every reaction of an animal comes from its cells; the outside environment may disturb those cells. Even the most quiet animal outwardly might be expected to reflect its own internal work, and the possible effect of a changed internal environment on a tissue specialized for irritability has equal possibilities, as the anatomical facts have proved.

Hence it is that one finds, and would expect to find, varied evidences of function in different 'normal' animals. The only result of the extraordinary function on this basis is to drive the cells further along in their phases of reaction, a quantitative difference in the sum total of reaction. All are in tone, many are already working,—to this is added more work. The mere existence of morphological differences within the same animal would be sufficient clue to something happening which needed to be correlated and interpreted, when it comes after any technic. Otherwise all cells would look exactly alike.

So, can one pick up any animal regardless of its individual existence, and use it as a control, expecting that it give necessarily a flat level of comparison against other animals of different habit, different experience? The true standard exists in the resting cell, a distinct morphological type, a constant species type (Dolley, '14). The only exact comparison between two individuals is in terms of the relative distribution of working to resting cells. Unless one recognize this, he will surely become involved in a maze of discrepancies.

It now only remains to explain why Kocher failed to find quantitative differences, as already noted in the citation, to nullify his criticism entirely.

Kocher made differential counts of the distribution of the stages in four dogs, one the undisturbed control, the others exercised one, two and a half, and five hours respectively. He says: "As will be seen in the table, the number of a particular type of cell varies considerably, but this variation is the same for the different animals." The understood conclusion is that all were on the same plane, even granting the existence of morphological types.

On scrutinizing Kocher's table 3, one is immediately struck by what may be a most significant point. Stage 6 stands conspicuous by its paucity, if not absence. Taking the counts from the worm of the cerebellum, he found none in the control animal, though identifying all succeeding stages. In forced activity, the greatest number thus identified was 2 out of 300 cells surveyed, while in the hardest worked animal none was found. Nor in the cord counts is he more liberal, three being the maximum found. Of course, in actual counts of 200 cells in a survey of 300, this may happen, but from my experience it is not so uniformly likely. The average run of stage 6, where all types are present, has been from 4 per cent to 10 per cent. For example, in the first series of counts published ('09 c), there was a maximum of 67 out of 600 cells actually counted (11 per cent), after six hours of exercise, 40 (6.6 per cent) after one hour, and a minimum of 25 (4 per cent), in a relatively very resistant dog in the effect displayed. A failure to identify stage 6 would dis-

turb a count quite considerably. What are the characteristics of
stage 6? Standing at the transition point between the shrunken
hyperchromatic Hodge stages and the following hypochromatism
and upset of the nucleus-plasma relation, it has a more swollen,
vesicular and disproportionate nucleus than the resting type,
though its plasma now comes to show the average distribution
of chromatic substance of that type. I have pointed out several
times that unless its nuclear size and appearance be kept in
mind, it will be mistaken for a resting cell.

A second point: Stage 13 is one of complete basic dechro-
matization. The Nissl substance is gone, likewise the nuclear
chromatin. The rapidity of such dechromatization depends on
the relative differentiation. It may appear within a few hours
in the Purkinje cell, though probably not unless the animal is
advanced in activity to start with. Not only has it never come
under my observation in a lower type of cell within the time
necessary to produce it in the cortex, but the indications have
always been that the lower cells at this time were many stages
removed from exhaustion. It was only marked, though still not
absolute, after two weeks of continuous excitation of the cray-
fish cell.

Yet Kocher is extremely liberal with stage 13. He always
finds it in the cervical and lumbar cord cells, and in two cases
out of the four animals counted there are more than from the
cerebellum. Not only do I regard this as impossible on the basis
of differentiation, but it does not jibe with the text, for he only
mentions grades of plasmic chromatolysis, which obviously is
another thing from nuclear plus plasmic dechromatization.
Nuclear *dechromatinization* would exact a comment from any one.
In other words, some at least of the stages identified as exhaus-
tion are fairly doubtful, and this carries closely related stages.
One is forced to the same deduction for Kocher's whole table 3.

It is the sort of rebuttal of a criticism that personally is very
distasteful, for it carries the possible imputation that the orig-
inator of said stages is the only one competent to pass judgment
upon them. This is not true, for eight students who have
worked with me have had no difficulty after several months

study in separating them as well as myself. As Kocher denies "progressive changes in the morphology of the cells," it is evident that he missed the finer points essential to a differentiation.

Outside of these technical points, no denial of the existence of quantitative differences can be made on the comparison of four animals. The range of individual variation is too great. There is no way of telling what the state of activity with which the experiment begins. Kocher's control animal may very well have been two or three times as functionally advanced as the one exercised the most was to begin with. I have seen several undisturbed animals who showed a degree of activity almost as great as one subjected to exhausting overstrain. The control comparison method, though valuable and frequently the only resource, affords no absolute deductions, unless all conditions are certain. Apparent inconsistencies, of which I have encountered many, one by one have cleared up as all conditions became known.

Just for one example, age is a factor. Very young animals usually show a hyperactive state as compared to the adult. Resting and early active cells may be absent in section after section. Very probably this is the reason why Kocher's three month old puppies showed "no discoverable differences in staining reaction."

One final rejoinder concerns a matter, which, though even more distasteful, I refuse to pass over. In April, 1910, I published the results of 2200 cell measurements. Even in the preliminary communication of November, 1909, on normal functional activity, which Kocher cites, the results from 1500 of these measurements were stated, which explicitly did not include those previously published from the shock and hemorrhage series. Further, in the same paper the results of differential counts of 3,600 cells were included. In still earlier communications, of April and July, 1909, on shock and hemorrhage respectively, which he also cites, it was made sufficiently clear that preliminary counts of 1300 and 1200 cells had been made, as it was stated that 100 cells were counted in each experiment.

From this brief survey, it may be imagined with what pained surprise one reads from Kocher, "Obviously the observations

were not over a large enough range of sections nor sufficiently controlled by actual counts of the various types of cells'' (p. 351). Kocher's work was finished in 1912, though the paper was not published until June, 1916 (see his footnote, p. 341). Before the end of 1911, I had published six papers, to four of which Kocher refers specifically in the text, and cites three in his bibliography —making an error in crediting authorship in that.

He then proceeds to juggle quotations to support his contention. As in any scientific writings, certain statements of small numerical amount, treating of finer detail or representing very preliminary work, are available. For example, he cites from my second paper (Journal of Medical Research, vol. 21, 104): ''Measurements were made of five cells of each type in two anemia experiments, one a fatal resuscitation, the other a repeated hemorrhage.'' Meagre data surely, and it reads as convincingly as a wilfully isolated text from the Bible. Only my next sentence, which he does not cite, happens to read: ''Since the results are the same as for shock, the number is considered sufficient for the present purpose,'' and the context goes on to enumerate the detailed identity. While not stated in words of one syllable, it conveys the impression to my mind at least of a constancy of dimension for each type, even for five cells. This is not all of the same thing, but it is enough. I leave the verdict to those disinterested.

EXPERIMENTAL DATA

In imitation of Kocher's experiment on normal activity, two puppies were chosen. They were females, from the same litter, weighing 2.7 and 2.5 kilograms, and a few days over three months old. One, the larger, was led on a fast walk over a country road course previously measured by a Stewart odometer on a Hudson motor car. It was desired to imitate Kocher's very fast pace of fifteen miles in three and one-half hours, but my two puppies had never been beyond the confines of the six foot square cage in which they were born and so lacked training. The animal trotted along willingly enough after it learned what was wanted of it, but though short rests were allowed, the pace was too fast, and before two hours it began to show distress. After two hours and ten minutes it refused absolutely to walk any further. The actually measured distance in my experiment was a trifle over six miles. It was then carried to the laboratory, and just as Kocher's dog, killed less than one hour after the exercise ceased.

TECHNIC

The unexercised animal then came into the experiment as the control, and every precaution was taken subsequently to preserve an exact identity of treatment. The two were simultaneously anesthetized with ether through the coöperation of an assistant, and killed by simultaneous bleeding. Their brains were removed at the same time so far as possible by duplicate motions, and the specimens from each dropped at the same moment, into the same fixing fluid, in a single container as follows: The bottles for each individual fluid used in fixing, dehydrating and imbedding were divided by a perforated partition into two parts and the material thus separated was subjected to identical conditions. Every transfer to the next solution was made by the simultaneous use of two forceps.

The fixing agent used was

	cc.
Saturated mercuric chloride	95
40 per cent formaldehyde solution	5

The material was then run through the graded alcohols,—30 per cent, 50 per cent, 70 per cent, 80 per cent, being iodized several days in the 80 per cent to remove the mercury, 95 per cent and absolute. It was then carried through xylol, xylol-paraffin, and two changes of 52° M.P. paraffin with the same precautions of identical handling. Finally the exercised and control tissue were inbedded side by side in one block.

The sections were cut by the same stroke of the knife at five micra in serial, and necessarily subjected to the same conditions of staining. As customary, the stain used was Held's erythrosin and toluidin blue.

Yet, save for a certain straining at a finicky precision, the procedure differed in no respect from previous ones, nor were the results in any way superior. Still Kocher is very harsh with me because "The control and fatigue material was handled entirely separately. Slight unavoidable variations in the exposure of the tissue to the various agents and different thickness of the cut sections would make such material worthless for comparative study." Surely not quite so bad as that. Bichloride is bichloride and alcohol is alcohol, and there are some of us who think that we get certain cell pictures because of the particular physico-chemical conditions in the cells, for we get them in the same animal by any fixing and staining reagent—and Kocher admits that he got them by his method. A microtome that can be depended upon to cut one micron serial sections, and there are

two in this laboratory, will surely cut sections at five micra as well tomorrow as today. Variations in section thickness are now and again unavoidable of course, but that is a negligible factor in median sections of a three dimensional and spheroidal body, which, excepting the eccentric nucleus, are the ones we use when plasma, nucleus, and nucleolus come into the same optical field. For, quoting a mathematical authority (see Dolley, '14) "the diameter of the cross section of a nearly spherical body varies very slowly for plane sections nearly median or diametral." Here is the mathematical reason why averages of individual
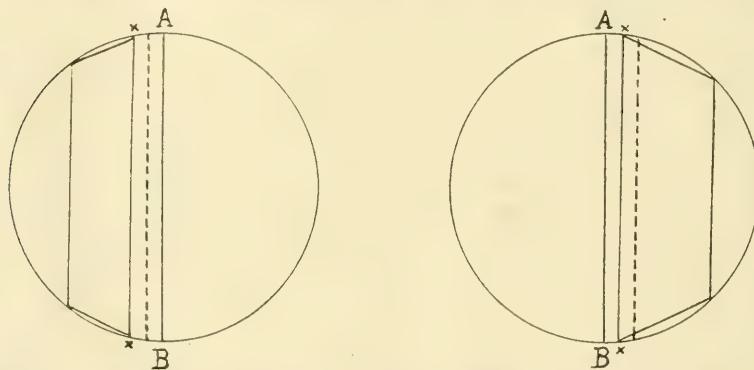
Fig. 1 Diagram of the relation of section frustra to the cell outline in the case of extra-diametral sections.

stages either of areas or diameters are dependable—they are from median sections with little variation from that.

The negligible effect of one micron variations in five micra sections may be illustrated very simply from the diagrams of figure 1. They represent two cells 20 micra each in diameter, which is the average for the transverse diameter of the Purkinje cell. The diameter AB is through the axial or median plane of each. Each section constitutes a frustrum and it is the edge of the maximum base of the section frustrum that we outline from the camera lucida. The frustrum in the left hand figure is a four micra, and the one to the right a six micra section, both being unfavorable possibilities outside of the true median section containing AB. The dotted line in each case marked a coin-

ciding five micra section. The points one would mark for the diameter in either case are marked x, and the slight deviation from the perfect five micra section indicated by the dotted lines as well as from the true median section containing AB is apparent.

THE COMPARISON BY DIFFERENTIAL COUNTS

For the technical interests of this paper only the Purkinje cell of the cerebellum is considered.

First it will be of interest to discuss the results of the differential counts which were made for the general comparison of exercise with the lack of it. The conditions under which both puppies had lived accounts to me for the striking difference that resulted from unaccustomed exercise. They were not merely rested up for a few weeks, for subsidence from activity of any degree goes most slowly (Dolley, 11a), but they had always lived under general conditions unconducive to wide activity.

TABLE 1
Differential counts of cells

	RESTING STAGE	STAGES OF ACTIVITY												UNCOUNTED CELLS	
	Stage 1	Stage 2	Stage 3	Stage 5'	Stage 5"	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11	Stage 12	Stage 13	Hyper-chromatic	Hypo-chromatic
Control animal.....	23	10	39	97	6	1	3	3	3	2	0	1	0	105	7
Exercised animal...	0	2	7	16	2	13	9	21	24	43	12	2	0	27	122

The data from the counts are set forth in table 1. The counts were made from paired simultaneously cut sections from the two cases. In each case a start was made from a corresponding corner and the line of Purkinje cells followed in continuity until all in the section were surveyed, and then, as the full quota was not made up in either case, one jump was made to corresponding corners of a pair the third removed in the serial to avoid counting any cell twice. Kocher's prerequisite of a complete nuclear membrane is as good a working basis as any, only it is to be remembered that if one admits hypochromatic cells whose karyosome is not visible, he can only approximate the exact stage

from stages 10 to 13, in which the state of the karyosome is the criterion. The karyosome is thus most rarely invisible. The cells which could not be exactly identified because not sufficiently in section were listed as hyperchromatic and hypochromatic, as has always been the practice. The differences in the counts submitted are as striking as in the diagnosed cells. Unless the differences are as marked as in the present case I do not consider a count of less than 200 actually diagnosed cells in imitation of Kocher as adequate, but the survey of 77 paired sections in this instance showed obviously that the general distribution was that of the count.

Table 1 may be summarized as follows: The transition to upset of the nucleus-plasma relation begins toward the end of the Hodge stage 5, actually with stage 5″. This is a convenient point of demarcation for comparison. In the control animal, 173 resting and early type cells, including stage 1–5′, are found, while in the other only 25 early type cells, with no resting cells at all, show the effect of exercise in driving cells beyond the early stages. On the other hand, there are only 19 cells in various stages of upset of the nucleus-plasma relation in the control as contrasted with 126 in the exercised animal. Taking the undiagnosed cells, the hyperchromatic ones belong between stages 1 and 5, the hypochromatic ones to the period of upset. The ratio in the control is 105 to 7, in the exercised animal 27 to 122. The quantitative difference between action and inaction is everywhere displayed.

It is also worth while to point out that there are no exhausted cells, namely, dechromatized in nucleus and plasma, in either case. The exercised puppy was not exhausted in the organic sense, but had much reserve. Its immediate distress, so far as it was nervous, came from the other side of fatigue, the waste product reaction. With rest and elimination, it could have gone on, as fits experience. Even the finding of a quota of exhausted cells does not indicate exhaustion of capacity—so long as there are other cells.

DIFFERENT METHODS OF MEASUREMENT AND THEIR IDENTICAL RESULT

Since this paper is primarily a comparison of technical methods, and since the morphological characters of the stages have been repeatedly summarized and will be again in a further paper to appear, it does not appear necessary to repeat them here. The stage numbers are uniform in all publications. The only chance of confusion may come in the combination of 4' and 5', 4" and 5" in tables 2 and 3.

TABLE 2

Average diameters of functioning stages in centimeters

CONTROL ANIMAL		NUMBER OF STAGE	EXERCISED ANIMAL	
Diameters of cell	Diameters of nucleus		Diameters of cell	Diameters of nucleus
6.86 × 3.43	2.38 × 1.59	1	6.91 × 3.44	2.40 × 1.67
7.57 × 4.46	2.65 × 2.16	2	7.42 × 4.35	2.61 × 2.08
6.84 × 3.80	2.29 × 1.74	3	7.25 × 3.69	2.11 × 1.51
6.43 × 3:31	2.25 × 1.29	4' and 5'	6.50 × 3.29	1.97 × 1.21
6.87 × 3.33	2.37 × 1.50	4" and 5"	6.82 × 3.71	2.36 × 1.70
6.96 × 3.49	2.50 × 1.76	6	7.14 × 3.79	2.48 × 1.87
7.23 × 4.07	2.49 × 1.93	7	7.81 × 4.13	2.45 × 2.01
8.23 × 4.32	2.59 × 1.99	8	8.43 × 4.44	2.47 × 2.14
7.73 × 4.91	2.52 × 2.22	9	8.47 × 4.98	2.49 × 2.21
8.33 × 4.90	2.51 × 2.28	10	8.34 × 5.09	2.53 × 2.30
		11	8.58 × 5.67	2.57 × 2.33

Stages 4 and 5 comprise the Hodge types, which were thus originally separated because stage 4 represents a more attenuated and smaller cell, and resulted in a somewhat different nucleus-plasma relation. In general, stage 4 now certainly has no special significance in itself, as a phase of immediate activity, but merely means a more slender cell to start with. It has, however, significance in that the slenderness is due either to lack of development or to functional atrophy. So for uniformity these stage numbers were kept, though in later publications, stage 5 has stood for the group. Then, after publishing in simple numerical order in a preliminary communication, it was found further that actually there was a transition type between

DAVID H. DOLLEY

TABLE 3

Size relations of functioning stages (Areas in square, volumes in cubic centimeters)

NUMBER OF STAGE	AREAS BY POLAR PLANIMETER		NUCLEUS-PLASMA COEFFICIENTS	RELATIVE VOLUMES AS CYLINDERS		NUCLEUS-PLASMA COEFFICIENTS	RELATIVE VOLUMES AS PARALLELOPIPEDS		NUCLEUS-PLASMA COEFFICIENTS
	Cell	Nucleus		Cell	Nucleus		Cell	Nucleus	
Control animal									
1	16.33	3.06	4.3	56.01	4.87	10.5	80.71	6.02	12.4
2	23.32	4.58	4.1	104.01	.9.89	9.5	150.57	12.36	11.2
3	19.42	3.30	4.9	73.80	5.74	11.9	98.77	6.92	13.3
4′ and 5′	14.57	2.30	5.3	48.23	2.97	15.3	70.44	3.74	17.8
4″ and 5″	15.46	2.82	4.5	51.48	4.23	11.2	76.18	5.33	13.3
6	16.69	3.42	3.9	57.25	6.02	8.5	84.77	7.74	9.9
7	20.25	3.85	4.3	82.42	7.43	10.1	119.76	9.28	11.9
8	22.91	4.10	4.6	98.97	8.16	11.1	152.59	10.26	13.9
9	25.56	4.44	4.8	125.50	9.86	11.7	186.33	12.42	14.0
10	27.72	4.61	5.0	135.83	10.51	11.9	200.00	12.86	14.5
Exercised animal									
1	16.45	3.21	4.1	56.59	5.36	9.6	81.77	6.69	11.2
2	23.11	4.31	4.4	100.53	8.96	10.0	140.40	11.29	11.4
3	18.66	2.58	6.2	68.86	3.90	16.6	98.72	4.81	19.5
4′ and 5′	14.80	1.88	6.9	48.69	2.27	20.4	70.36	2.88	23.4
4″ and 5″	17.16	3.02	4.7	63.66	5.13	11.4	93.97	6.82	13.1
6	19.14	3.77	4.1	72.54	7.05	9.3	102.56	8.67	10.8
7	22.02	3.93	4.6	90.94	7.90	10.5	133.21	9.90	12.5
8	25.59	4.29	5.0	113.60	9.18	11.4	166.18	11.31	13.7
9	28.23	4.35	5.5	140.59	9.61	12.6	210.06	12.16	16.3
10	30.08	4.74	5.3	153.11	10.90	13.0	216.07	13.38	15.1
11	34.04	4.87	6.0	193.01	11.35	16.1	275.84	13.95	18.8

the strict Hodge type, stage 4 and 5, with its dense crenated nucleus and stage 6 with its edematous nucleus, to which nuclear transition the plasma corresponded with an intermediate condition. Rather than change the numbers, this transition stage has been indicated since as 4″ and 5″ to distinguish it from the strict Hodge type, 4′ and 5′.

Taking up the problem of measurements, it is obvious that with the cell aggregate distributed as a number of distinct types or stages, each of a certain significance, one thing to do is to

measure these types selectively to determine their absolute and relative sizes. This is what the writer has always done, and it will be clear enough from the sort of information it affords that it is the one thing to do. Another thing which might be done, as Kocher did, and which has always been done by other investigators, is to lump all cells irrespective of type and strike an average. The uncertainty of this method, which may or may not give true results, will be very easily shown through the data obtained by stage measurements. The employment thereof is the one reason for the wide variety of results which has been obtained by different workers.

The stage measurement is frankly a selective measurement, but in the sense of the selection of types as they come, not of picking cells here and there according to their probable suitability to work out right. It can be made, and has been made, as rigid in requirement as the aggregate way can be. The cerebellum is peculiarly suited for this because one can start at a convenient point and follow the line of Purkinje cells around with no danger of doubling back until all have been covered. If one then exacts the requirement to measure and diagnose according its stage every cell in such a median section that its karyosome and the trunk of its dendrite are included, he has no more leeway than by the aggregate method.

The requirement of the trunk of the dendrite is specified for the following reason in the case of the Purkinje cell. It is known to be pear-shaped, which I have confirmed by some fifty reconstructions in wax. I am not going to measure such a cell unless it is obvious that it is fairly complete in the plane of its longitudinal axis, for it would be averaging a spheroidal dimension with elongated ellipsoidal ones.

With a greater or less unequal distribution of stages, the quota of a uniform number for each stage will be filled up at different points, one by one all becoming full. One simply proceeds from section to section, passing over those stages whose quota is complete, but adding to the incomplete as they come. Of course this will demand many sections when a stage is scanty, —for the almost absent resting cells in the exercised puppy it took 77. One loses the benefit of that "cut-with-one-stroke-of-

the-knife," but in view of the explanation made and the results to be shown, this is negligible.

Twenty cells were measured to a stage. There were thus 200 cells (10 stages) measured from the control and 220 (11 stages) from the exercised animal. Stage 11 was not measured in the control for the simple reason that none was found.

The procedure was to outline the accepted cells one after another by means of the camera lucida (Zeiss). The lens system used was the Zeiss comps. oc. 12, homo. 2 mm. oil immersion, tube length 153 mm., X 1960 at stage level. The magnification was adjusted to conform to some previous measurements, and it is believed that the area determined from larger magnifications is less subject to small mechanical errors. At the point where the dendrite becomes of uniform size the cell outline was closed. When the full set was outlined, the area in square centimeters was read for each cell from the polar planimeter. At the same time, the maximum longitudinal and transverse diameters of cell and nucleus were measured in one-half millimeters, to compare and test the accuracy of this previous method. The areas and dimensions were then averaged for each stage set. The average diameters are set forth in table 2 and the average areas in table 3 under that head.

From the average diameters the average relative volumes of cells and nuclei were estimated. The third dimension being unknown, it can only be approximated on the basis that it will average the same as the transverse diameter—another thing that individual cell reconstructions bears out. The term relative volume is used because the volume was calculated as that of a parallelopiped, namely, the length by width by depth—the longitudinal axis multiplied by the square of the transverse. Mathematically, the formula for such elliptical bodies is $\frac{2}{3}$ [2a.-π. (b)^2], in which a and b represent the major and minor radii. For the sphere it becomes $\frac{4}{3}$ (π r^3).

If the ratio between two such bodies whose radii are a and b and a' and b' respectively be expressed as a fraction, it becomes on cancelling out the common factors $\frac{a\,b^2}{a'\,b'^2}$, or the diameters

themselves may be used. The relative volumes as parallelopipeds thus obtained are set forth in table 3.

The cell volume less the nuclear volume gives the plasma volume. The plasma volume divided by the nuclear volume gives the nucleus-plasma coefficient—the size factor of nucleus to plasma. The area relations of nucleus and plasma were also computed. The plasma volumes are not stated, but the nucleus-plasma coefficients appear in table 3.

Between the area method and the diameter method a third combination is possible. The planimeter gives the area of a roughly elliptical median plane of the cell. Multiplying this area in each stage set by the corresponding transverse diameter of the cell and nucleus gives another set of data of relative volumes. This merely represents the volume as contained within a cylindrical surface instead of a parallelopiped, and the differences are in proportion, but there was a curiosity to see how it would work out. These figures are the middle set in table 3.

A graphic representation of the data of volumes and nucleus-plasma coefficients gives the most convenient basis for technical comparison. Instead however of publishing the three sets each of size (area and volume) and nucleus-plasma curves from each animal, only the three size curves of the control after the three methods (fig. 2) and the three nucleus-plasma curves of the exercised one (fig. 3) are presented. A reference to table 3 will show that the trend of the counterpart figures is identical in the two cases.

The reduction of the area and volume figures for charting was made in terms of the ratio of the resting cell body to its nucleus. That is, in the case of the area figures, the ratio of stage 1 is 5.3 : 1; the whole series of cell areas was divided by 5.3, the nuclear figures by 1. This procedure has the value of making the curves represent not only absolute size in the ordinates for each stage, as would be obtained by any convenient divisor, but also of giving the relative size of each succeeding stage to stage 1. Further, since this procedure makes the cell and nucleus start from the same height of ordinate, the shifts of relation between cell body and nucleus for each stage are shown. It gives in assc-

1   2   3   5′   5″   6   7   8   9   10

Fig. 2 Size relations of function compared by the three methods.
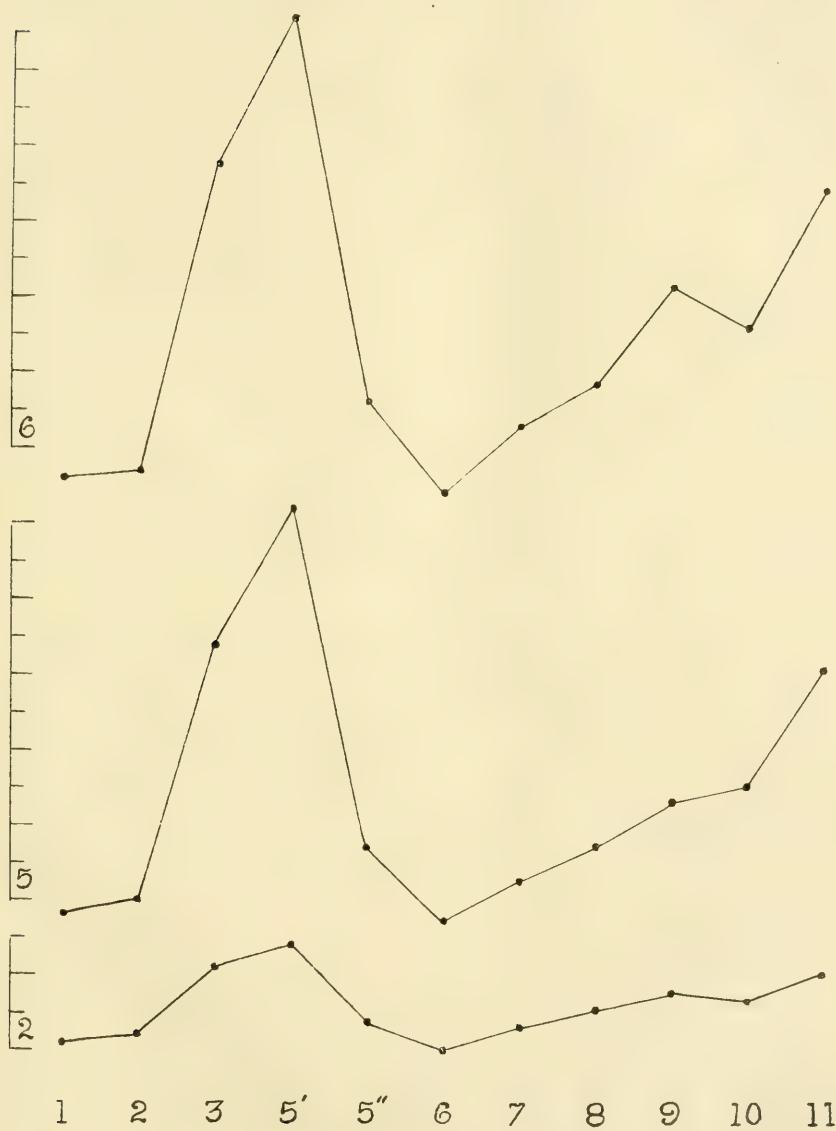
Fig. 3   Nucleus-plasma relations of function compared by the three methods.

ciation with volumes then a graphic representation of the nucleus-plasma relation.

In order to get all three graphs of figure 2 on the same page the cell and nuclear figures were each further reduced one-fourth. The lower graph is the area, the middle the relative volume as a cylinder, the upper the relative volume as a parallelopiped. The solid lines are cell, the broken lines nuclear sizes in each case. As the relative sizes to each other by the three methods have no significance, the graphs are conveniently placed one above another, and their abscissas omitted for simplicity.

The three nucleus-plasma curves from the exercised animal are made by plotting the coefficient figures for each set (table 3), reduced one-half, as ordinates above a base line. They are likewise placed one above another in figure 3 for easy comparison, irrespective of their comparative heights, and their abscissas are omitted. The comparison is with the resting cell in each case and not in terms of absolute values. However, the interrupted scale to the left shows in centimeters the actual height for the one-half reduction of each curve.

The technical methods may now be compared at a glance. The planimeter or area method affords results that are absolutely identical in every detail with the diameter method, both in size and in nucleus-plasma relation. Note the crossings of cell and nuclear lines in figure 2. Even the slight variation from the usual steady upward trend from stage 9 to stage 11 in the nucleus-plasma curve shows up in area and parallelopiped. Previous results by the diameter method are merely confirmed, no more, no less.

The planimeter or area method alone, therefore, has no special or superior value. True, it gives the exact areas of any section through cell and nucleus. It is a valuable check on the diameter method, particularly in the case of irregular cells, but the irregularity makes consideration of their three dimensions essential.

On the other hand, exclusive use thereof, in my opinion, would tend to make one think in terms of two dimensions, as Kocher did, for his only reference to a third dimension is found in the

heading of his table 2, "Volume expressed as square inches," a very *plane* conception of volume.

The area method necessitates the same resource to averaging. Where relative sizes are all-important, the smaller variations inherent in the two dimensional measurements as compared with the corresponding augmentation of these differences in greater and greater degree in three dimensional calculations is quantitatively misleading and may cause important points to be minimized. Take 2 x 2, and 3 x 3; one is 4, the other 9; but 2 x 2 x 2 is 8, and 3 x 3 x 3 is 27. Here is another fault in all size comparisons in the literature which makes them less productive—the diameters alone were used (compare tables 2 and 3). Mathematically stated, in a series of increasing squares the first differences are in arithmetical progression, in one of cubes the second differences are in arithmetical progression.

Along with the confirmation of previous technical methods certain important conclusions are corroborated by the added data.

In the first place, the nucleus-plasma coefficients of the resting cell (stage 1) are 12.4 for the control, and 11.2 for the exercised animal by the diameter method, and 4.3 and 4.1 respectively by the area method. The average resting cell coefficient so far is 11.7 by the diameter method, and the range of deviation is 11 to 12.4. The two figures above, 12.4 and 11.2, fall therefore within this range. Two additional individuals conform to the law, of species identity of the nucleus-plasma norm (Dolley, '14).

In the second place, it may be noted that the coefficient figures of stage 2 do not vary more than those of stage 1 just discussed —12.4 to 11.2 and 11.2 to 11.4. In short, as has always occurred in stage 2, though the size undergoes a 50 per cent or greater increase, the nucleus-plasma relation remains constant. This is most important for the deduction of an exact proportionate increase of nuclear and plasmic materials in the beginning of activity, a purely quantitative increase of the same materials in each element.

The third point is the close identity of area and volume for the resting cells in the two cases—16.33 and 80.71 with 16.45 and 81.77. What does it probably mean? The deduction has

been made in previous work ('14, p. 494), and more strongly supported in some unpublished work, that function is the sole determinant of absolute size. Non-divisional growth in mass is a functional growth. Here are two animals born together and living under functional conditions as identical as may be. Their cells show the same absolute size. It is a noteworthy verification of the deduction.

It fits in with this relation of function to size that the evidence is accumulating of a tendency to a uniformity of absolute size among corresponding nerve cells of animals of the same species. When sufficiently demonstrated it would be understandable on the basis of average general functional usage. The exceptions thereto so far in the dog, the unusually large cell, have been associated with a known history of unusual training and activity. It makes the nerve cell agree with Conklin's conclusion that within the same species cell size is approximately constant. Making simply a statement here of the probable principle, it is to be noted that these two dogs, being not yet grown, offer no evidence for or against species uniformity of absolute size, save that they are progressing together under identical conditions.

It might be expected and to some extent it is true that all stages succeeding stage 1, being based quantitatively upon it, might show this same correspondence of absolute size. However, in all stages except stage 1, one encounters a shifting range of size throughout the stage. The results will vary according as the majority of cells are at one end or the other, or well distributed in the chance of a section. Stage 1, though there are intermediate grades to stage 2, was frankly selected in both animals as the nearly flat type, with this very point in view, and intermediate stages were thrown to stage 2.

THE INCONSTANCY OF COLLECTIVE AVERAGES OF FUNCTIONING CELLS

It only remains to demonstrate from the data in table 3 the inconstancies which may result from averaging all cells irrespective of their functional state, and to expose the fallacy of deny-

ing on such a basis the existence of functional size changes.
For the inconstancies, take the control data: The average area
of thes mallest cell, stage 5'—and the average covers its own varia-
tions—is 14.57, that of the largest is 27.72 or nearly double;
the average volume of the smallest cell is 70.44, that of the
largest 200 or nearly triple the size. In the exercised animal
with the still larger stage 11, the largest volume is nearly quad-
ruple the smallest, and its area again more than double. Is it
not apparent to any one that if such widely variant sizes or areas
are averaged, the result depends upon the particular distribu-
tion of types and that a wide range of results is possible? If,
out of 20 cells, even in area computation, 5 measure 14 sq. cm.
and 15 measure 27, the average is 24, whereas if 15 measure 14,
and 5 measure 27, the average is 17 sq. cm. The results may
or may not prove anything about the immediate functional state.

One can then with fair probability explain what did happen in
Kocher's case. He finds only small variations in average area
size between control and exercised animals and these not con-
stant. So far as different functioning stages appear, they tend
to be distributed rather than bunched. A general average, taking
into account the smaller range of variation of area figures, would
tend to equalize the differences due to unequal distribution of
various-sized types.

So Kocher, having smoothed out individual cell variations by
averaging, found no great difference between an animal and its
control. His results are just what might be expected in prob-
ably the majority of cases, and instead of confounding the writer
in respect to functional size changes, tend only to support the
induction previously stated of a uniformity of cell size as a gen-
eral rule for a species. Were it not for this tendency to equality
of size of corresponding cells, collective averaging would not have
afforded so many positive results as it has.

Since the method of collective averaging is the one which has
been always used, how about such results as those of Hodge?
Are they discredited? No, but they must be qualified. Hodge
found a smaller size in the stimulated spinal ganglion as com-
pared with the unstimulated simply because there were enough

smaller cells to bring the average down. In such a slowly re-
acting type morphologically, it would be the usual result for a
certain period, but not indefinitely. Prolonged stimulation
would be driving the cells after they reach this point of shrink-
age to enlargement—and enough of these larger cells would first
equalize the comparison and then bring a larger size in the stimu-
lated cells. So Kocher did not with entire consistency obtain
Hodge's results. Yet as a matter of fact, accepting the smallest
differences, he did duplicate Hodge's results in eleven out of
fifteen series from the spinal cord and associated ganglia. Fur-
ther, his exceptions and the trend of his figures follow to a con-
siderable degree the explanation given.

To sum up for collective averages, variations may indicate
first the predominance of related types of present function.
When they do, the variations may be above or below the mean,
depending on what sized types predominate. No variations will
show up in certain distributions of types. Second, variations
may indicate an acquired state of functional hypertrophy, which
has nothing to do with the immediate function, but which, as
every one should know, may enter as a condition and not a the-
ory for the specialized cell. When this complication is introduced
it may combine to lessen, equalize or exaggerate the other possi-
bilities of variations from the first group of immediate function.
The functional hypertrophy has an opposite possibility, the func-
tional atrophy. Why waste any more time over this method?
It gave of what it has to the pioneers. It is a scientific solecism
that function, the one faculty which results from the differen-
tiated state, is the one and only factor which has been neglected
in making cell measurements on differentiated cells.

If any one wishes to investigate the size changes of function,
he must identify and measure functioning stages and the rest-
ing type from which they spring. This will give him function
in itself. The resting cell and that alone will give him species
size, its mean and its variations, species relativity of plasma to
nucleus, and a basis of comparison between individuals uncom-
plicated by the degree of present function. For species size,
once the mean is determined, the variations may be analyzed,

and all complications of comparison sufficiently discounted. For species relativity of plasma to nucleus, a constancy can not be denied until it is analyzed apart from immediate function. This applies not only to the nerve cell, but to all specialized cells in their proper measure. Until that be done, criticisms of Richard Hertwig's nucleus-plasma relation, of which the species constancy is an extension for the nerve cell, are not worth the paper on which they are written. When it is done, there is still no reason to this investigator to doubt that the nucleus-plasma relation will be a fact and no longer a theory.

CONCLUSIONS

The planimeter or area method applied to the stages of function affords results which are identical with those from the diametral method of measurement, both in size and in nucleus-plasma relations. Previous conclusions for functional size changes and species size relations of nerve cell bodies are verified.

The value of the planimeter method is as a mutual check on the diametral method, particularly in irregular cells. Alone it has no special or superior value, while it gives only two dimensions, with smaller variations than in volume calculation, and is quantitatively misleading.

Collective averages of cells irrespective of their functional state, which has been the usual basis of comparison between individuals, afford inconstant results and should be discarded. It is a scientific solecism that function, the one faculty which results from the differentiated state, is the one factor which has been neglected in the measurement of differentiated cells. To deny functional size changes on this basis because of small and inconstant variations between one animal and another, as Kocher has done, is a fallacy, and such results indicate only the tendency to a uniformity of absolute species size for corresponding nerve cell bodies.

BIBLIOGRAPHY

DOLLEY, D. H.   1909 a   The pathological cytology of surgical shock.   1.   Jour. Med. Research, vol. 20, 275.

1909 b   The morphological changes in nerve cells resulting from over-work in relation with experimental anemia and shock.   Jour. Med. Research, vol. 21, 95.

1909 c   The neurocytological reaction in muscular exertion.   Amer. Jour. Phys., vol. 25, 151.

1910   The pathological cytology of surgical shock.   11.   Jour. Med. Research, vol. 22, 331.

1911 a   Studies on the recuperation of nerve cells after functional activity from youth to senility.   Jour. Med. Research, vol. 24, 309.

1911 b   The identity in dog and man of the sequence of changes produced by functional activity in the Purkinje cell of the cerebellum. Jour. Med. Research, vol. 25, 285.

1914   On a law of species identity of the nucleus-plasma norm for nerve cell bodies of corresponding type.   Jour. Comp. Neur., vol. 24, 445.

KOCHER, R. A.   1916   The effect of activity on the histological structure of nerve cells. Jour. comp. Neur., vol. 26, 341; Jour. A. M. A., vol. 67, 278.

THE FOREBRAIN OF ALLIGATOR MISSISSIPPIENSIS

ELIZABETH CAROLINE CROSBY

From the Anatomical Laboratory of the University of Chicago

FORTY-SIX FIGURES

CONTENTS

The olfactory system is highly developed in the reptilian forebrain. Not only are large basal nuclei present, but there is clearly differentiated cortex and all the important olfactory tracts of higher forms are represented. The larger part of the forebrain, then, is concerned in the reception of olfactory impulses and their correlation with incoming diencephalic impulses of various sorts. In fact, the diverse distribution of the incoming diencephalic tracts, each of which has its own characteristic functional significance, has been one of the prime factors in the differentiation of the forebrain into its various cortical and basal centers. Although the greater part of the reptilian forebrain is under the influence of the olfactory fibers, there is a considerable portion which receives a much smaller number of these fibers and is dominated by the ascending fibers from the somatic nuclei of the thalamus. Through these somatic

diencephalic fibers, the functional motive for the formation of the corpus striatum and the neopallum has been introduced into the hemispheres and in the alligator forebrain these somatic centers are already beginning to take form and to establish certain characteristic and fundamental relationships which will be discussed later.

The specific distribution of the olfactory and the non-olfactory fibers, the positions and the relations of the various centers and, finally, an analysis of these data in terms of their functional significance, these are all essential to the adequate understanding of the morphology and the evolution of the forebrain. The present report is concerned with a description of these centers in the alligator forebrain and of the fiber tracts which put these centers into relation with each other and with the diencephalon. Finally, an attempt has been made to effect a partial correlation and interpretation of the factual data obtained.

The advice and assistance of Dr. C. Judson Herrick have made possible whatever there may be of value in these notes. For these things and for the opportunities accorded me in his laboratory, I wish to thank him most sincerely. I am indebted to Miss Jeannette B. Obenchain for assistance in technique and to other members of the Department of Anatomy of the University of Chicago who have given helpful suggestions. Mr. Streedain has very kindly made the drawings of the gross material and has lettered the drawings of the microscopic material and otherwise aided in preparing them.

MATERIALS AND METHODS

The animal chosen for study was Alligator mississippiensis. The individuals were small, varying in length from 30 to 55 cm. The drawings of the surface anatomy (figs. 1 and 2) were made from the brain of a 55 cm. alligator.

The silver impregnation methods of Golgi and Cajal and the toluidin blue method were the chief ones employed. Two rather imperfect series, one stained with Ehrlich's haematoxylin

and the other by the Leuden van Heumen method, were used
to check certain fiber tracts, but the paths described in this
paper are almost entirely those brought out by the method of
Cajal. These series were further supplemented by a trans-
verse series stained with carmine and a second such series stained
with haematoxylin (both the property of Dr. C. J. Herrick).
One of the Cajal series was very kindly loaned by Dr. P. S.
McKibben.

Several specimens were stained by various modifications of
the Weigert method.   While the results were very satisfactory
for the study of parts of the brain below the thalamus, these
preparations contributed little of value in the study of the con-
nections of the cerebral hemisphere because practically none of
the fiber tracts in this part of the brain at the ages here inves-
tigated have become myelinated.

HISTORICAL NOTES

Rabl-Rückhard ('78) gave an excellent description and some
very clear pictures of the gross appearance of the brain of the
adult Alligator mississippiensis. A brief description of the ex-
ternal form and some details of the microscopic anatomy of
the same species were given by C. L. Herrick ('90), based upon
young specimens under 45 cm. long. Figures of the alligator
brain are given in Wiedersheim's Comparative Anatomy and
other figures and descriptions of the external form are scat-
tered throughout the literature and it is unnecessary to enter
into a detailed account of the gross relations, the essential fea-
tures of which are shown in figures 1 and 2. DeLange ('11)
presents a series of photographs of surface views of reptilian
brains, among which are those of Alligator sklerops, and in a
later paper ('13) the same author publishes a series of twenty
sketches of cross sections through the thalamus and the mid-
brain of this species. Unger ('11) has given a brief account of
structure and fiber tracts of the forebrain of young specimens
of Alligator lucius and Crocodilus niloticus which is preceded
by an excellent summary of previous work on the forebrain of
the Crocodilia.

Many workers have indicated the presence of an epiphysis in the alligator brain. The work of Albert Reese ('10) has shown the structure so named is a paraphysis and that no epiphysis is present in the alligator, even in the embryo. In 1908, Reese published an account of the general embryological development of Alligator mississippiensis.

No attempt will be made to review systematically the extensive literature on the brains of reptiles in general, though references to this literature will be made as occasion may arise. Among the classical descriptions of the reptilian brain especial mention should be made of the valuable description and figures of the turtle brain published in 1895 by Mrs. Susanna Phelps Gage, in commemoration of whose important contributions to comparative neurology the current volume of The Journal of Comparative Neurology is dedicated.

<center>CELL STRUCTURES</center>

The positions of the nuclei of the telencephalon, with such details of their cell arrangement and cell structure as have been observed, will first be described, together with some more fragmentary observations on the diencephalic nuclei. Then using these facts for orientation, the courses and connections of the fiber tracts will be considered.

<center>*Olfactory Bulb*</center>

Johnston ('13) described the presence of a nervus terminalis in the reptiles. The preparations available are not suitable for the identification of this nerve in the alligator.

The cell bodies of the peripheral olfactory neurones lie in the olfactory epithelium of the nasal cavity. Their axones pass back as fibers of the olfactory nerve or 'fila olfactoria.' These fibers are unmyelinated and, after a very short course, enter the olfactory bulb. In its outer portion they break up into terminal arborizations which form synapses with dendrites of the mitral cells (figs. 23 and 24) and with other receptive cells of the olfactory bulb.

These places of synapse are called glomeruli and, scattered among these glomeruli, are a number of small cells which send their dendrites and, probably, their axones (though there is no proof in the material used for this statement), into the various glomeruli and so serve for the correlation of impulses. These are the type that Cajal calls intraglomerular cells.

Mitral cells (figs. 23 and 24). In transverse sections, the mitral cells have a ring-like arrangement around the granule cells as a center (fig. 13). Near the anterior end of the bulb they form a somewhat diffuse mass but soon take on their characteristic arrangements. They are replaced by other cell groups in the olfactory crus.

In the ventro-medial portion of the bulb, near its anterior end, the mitral cells form a curious depression or 'fossa.' This 'fossa' was first described by C. L. Herrick ('90), who said that a separate slip of the olfactory tract arises from it. The 'fossa' is very evident in both the toluidin blue series and those series prepared by the Cajal method. In the latter series, the fibers can be seen passing caudad from it and forming a part of the tractus olfactorius. There is a special thickening of the glomerular layer in that region, which pushes the mitral cells inward and causes the depression. Beyond being a point of entrance for a particularly large number of olfactory fibers, it does not appear to have any special significance.

A study of the toluidin blue preparations shows a considerable variation in the shape and size of the different mitral cells. On the whole, the nuclei tend to be rather large and are usually placed nearer the ventricular border of the cell. An abundance of Nissl substance is present in the cytoplasm.

The variations in size among the mitral cells are brought out most clearly in the Golgi preparations. Some of the different types observed there are illustrated in figures 23 and 24. Round, stellate, and large pyramidal forms are seen. A mitral cell usually has two main dendrites and several smaller dendritic branches. The larger dendrites are thick and thorny and enter into the formation of glomeruli with the incoming olfactory fibers. The smaller dendrites extend as far outward as the

glomeruli but have never been observed entering into the formation of a glomerulus. They intermingle with the dendrites of other mitral cells and granule cells and so make one of the important elements of the plexiform layer. This plexiform layer, then, provides an additional mechanism for the increasing and the summating of stimuli.

The axones of the mitral cells arise from their ventricular border. A short distance from the cell body, the axones of the larger cells divide into two main branches of approximately equal size. One branch enters the granule cell layer and comes into synaptic relations with its neurones. The other branch runs caudad in the tractus olfactorius, giving off, at various levels, numerous fine collaterals into both the granule cell and the plexiform layers. The first branch and fine collaterals of the second branch are chiefly (although not entirely) to provide a mechanism for the summation and strengthening of stimuli. The main part of the second branch provides for the conduction of the impulse to the secondary centers. In some of the mitral cells apparently only one of the branches may be present. When this is the case, it is usually the one into the tractus olfactorius which is represented.

Granule cell layer (figs. 13 and 25 to 28).⁺ The inner granule cells occupy the inner portion of the bulb about the ventricle, next to its ependymal lining. Extending caudad, at about the beginning of the olfactory crus, this layer is replaced by the cells of the nucleus olfactorius anterior, although the material at hand has not permitted the drawing of so precise a line of demarcation as Johnston ('15) is able to do in Cistudo carolina.

In teleosts, Sheldon ('12) has called the whole mass a part of the nucleus olfactorius anterior and then explained that certain neurones function as granule cells. The conditions found in the alligator represent an advance in differentiation over those described for teleosts; yet even here it does not appear that the granule cell layer is so physiologically distinct from the nucleus olfactorius anterior since, in part, its cells still serve as secondary olfactory neurones.

The granule cell layer shows a wide range of types among its neurones. The following types, based on a study of Golgi preparations, have been distingushed among the granule cells of the bulb.

1. Intrinsic or type II cells. These neurones have small cell bodies, with dendrites that are short, thorny, and branching, and which pass out in every direction from the cell body. No axones can be distinguished. These cells are intrinsic neurones, serving for the correlation of impulses within the layer. Some of the smaller stellate cells appear to serve as intrinsic neurones, at least so far as can be judged from the material studied.

2. Stellate cells (figs. 27, 28). These are similar in appearance to the cells so named by Sheldon in the teleostean olfactory bulb. The cell bodies are angular or somewhat star-shaped as the name indicates. The dendrites are thick and thorny and many branched and extend out towards the periphery of the bulb. In the plexiform layer they interlace with the dendrites of the mitral cells and of the goblet cells. Some of the dendrites extend outward into the glomerular layer but it was not determined whether these dendrites actually entered into the formation of glomeruli, as Sheldon ('12) found to be the case in the teleosts. The axones in many cases form synapses with branches of the mitral cell dendrites. Sometimes they enter the tractus olfactorius, although they have been followed no great distance in it. Some of the smaller stellate cells do not send their dendrites outward beyond the cell bodies of the mitral cells. Furthermore the axones of such cells often end about other cells of the bulb and so serve as intrinsic neurones.

3. Goblet cells (figs. 25 to 27). These are large, oval cells whose dendrites are similar in appearance to those of the stellate cells. Sometimes the dendrites of the goblet cells reach the glomerular layer, and have been seen entering into the formation of a glomerulus. In other cases, the dendrites of the goblet cells do not enter the glomerular layer but are dependent upon the mitral cells for their stimulation. The axones of the goblet cells enter the tractus olfactorius, at least in some cases.

From the standpoint of their types of synaptic connection apparently three functions are served by the neurones of the granule cell layer. The first of these is that of diffusing and summating the incoming olfactory impulses and so strengthening the discharge into the hemispheres. This purpose is served by the type II cells, the stellate cells, and, in part, by the goblet cells (particularly those found in the anterior part of the bulb). All these cells receive their impulses by way of the mitral cells and do not send their axones into the tractus olfactorius.

A second group of these stellate and goblet cells send their dendrites into the glomeruli and their axones into the tractus olfactorius and so, from a functional standpoint, are practically mitral cells.

The third function served by neurones of the granule cell layer is that of acting as the cells of secondary olfactory nuclei. Such cells receive impulses from the mitral cells and send their axones into the tractus olfactorius. The goblet and stellate cells offer examples of this type of neurone.

Judging from what is known of the development and specialization of the centers of the central nervous system, it seems but fair to suppose that, in phylogeny, the centers of the olfactory bulb arose from undifferentiated central gray. Johnston ('98) has shown that in Petromyzon and in Acipenser, neurones of this mitral cell type are found all through the central gray. The same author ('15) has described, in Cistudo carolina, a granule cell layer in which are cells functioning as mitral cells.

Certain cells of the central gray (on the whole those nearer the periphery) will receive a larger number of the incoming olfactory impulses. Under the operation of neurobiotaxis (Kappers, '14) such cells will be drawn toward the periphery and, in this way, a mitral cell layer will be formed. Accompanying such a migration toward the surface and the consequent higher specialization, there will be a differentiation in form and in size to meet the greater demands.

Not all the cells left in the central gray will lose their connection with the fila olfactoria and so certain goblet cells and probably some of the stellate cells (although the proof for this is not

absolutely clear) found in the olfactory bulb of the alligator illustrate this condition, for they lie in the granule cell layer— the position of the primitive central gray—yet aid in the formation of the glomeruli and send their axones into the tractus olfactorius. Sheldon ('12) has shown a similar condition in his description of certain stellate and goblet cells in the olfactory bulb of teleosts.

Other stellate cells and many of the larger goblet cells of the reptilian granule cell layer have lost their connection with the fila olfactoria and receive their impulses by way of the axones of the mitral cells. In turn, they send their axones through the tractus olfactorius and, from a functional standpoint, are secondary olfactory neurones. A similar state of affairs has been described by Sheldon ('12) for teleosts.

A smaller number of small stellate cells and goblet cells have lost their connection with the tractus olfactorius and discharge into the plexiform layer, serving apparently the usual correlating and summating function of granule cells. Are these the forerunners of the most highly specialized granule cells of higher forms?

Olfactory crus

The anterior continuation of three of the centers of the hemisphere are to be found in the crus (fig. 14). These are the nucleus olfactorius anterior, the pyriform lobe complex, and the hippocampus. Mitral cells are found in the olfactory bulb back to the point where it passes over into the crus. There they are replaced on the lateral side by cells of the pyriform lobe complex and on the medial side by the anterior continuation of the hippocampus. In the anterior end of the crus, the granule cell layer is replaced by the cells of the nucleus olfactorius anterior, which takes its characteristic ventro-medial position. The position, cell structure, and significance of these centers will be discussed immediately under the head of centers of the hemisphere.

Centers of the hemisphere

Both basal and cortical centers are found in this region in the alligator. The former will be described first. The term olfactory lobe will be used a number of times in the following description. By that term is meant the anterior portion of the hemisphere, including the secondary olfactory nuclei from the posterior end of the crus to the beginning of the primordial general cortex.

Nucleus olfactorius anterior (figs. 3 to 5 and 14). In the crus the nucleus olfactorius anterior occupies a ventro-medial position. It extends back into the hemisphere and runs caudad for some distance.. It is gradually pushed away from the surface by the cortical layer of the tuberculum olfactorium and is directly continuous with the non-cortical part of that nucleus. Throughout most of its extent in the hemisphere the boundary between it and the anterior end of the caudate nucleus can not be defined. Johnston ('15) has described this nucleus in the turtle. He considers, however, that it makes up, for the most part, just the head of the caudate nucleus.

So far as has been observed in the preparations made after the Golgi method, the cells of the nucleus olfactorius anterior are round or goblet shaped and are comparable in form and general appearance with the goblet cells of the granule layer of the olfactory bulb. Figure 29 shows a goblet cell of this nucleus.

This nucleus is a secondary olfactory center, receiving impulses by way of the tractus olfactorius and discharging, through the axones of its cells, into the tuberculum olfactorium, the parolfactory region, and the hippocampus.

Area parolfactoria (figs. 7 to 9, 16, 17). The cell groupings found in the ventro-medial wall of the hemisphere in lower vertebrates have caused much discussion. Meyer ('95), Unger ('06), C. J. Herrick ('10), Johnston ('13 and '15), and a number of other observers have mapped out the cell groups in this region. They have not all agreed in regard to the embryological and phylogenetic significance of these cell masses and as a result a somewhat confusing nomenclature has appeared.

In his 1913 paper Johnston has discussed fully the relative positions and extents of the cell masses in this ventro-medial region of the hemisphere in cyclostomes, selachians, reptiles, and monotremes. Among the reptiles, he has described particularly the conditions found in the turtle. In regard to the alligator he says ('13, p. 387), "In Alligator mississippiensis the features described above (that is the relations of the primordium hippocampi and the parolfactory area in the turtle) are repeated so exactly that it is unnecessary to present separate drawings. There are differences in general form, and the area parolfactoria is relatively smaller than in the turtle."

In the turtle Johnston has pointed out the presence of a primordium hippocampi, ventral to the hippocampal formation. A similar primordium is present in the alligator and below this primordium and separated from it by a cell free zone and, in turtles, by a sulcus limitans hippocampi (Johnston, '13) is the area parolfactoria (in part, Herrick's septal area). This parolfactory area is divided into medial and lateral portions which, following Johnston ('15), have been termed in this account the medial and lateral parolfactory nuclei respectively. This area parolfactoria is not the lobus parolfactorius of Edinger.

Medial parolfactory nucleus (figs. 7 to 9, 16, 17). This nucleus consists of cells of the more medial part of the parolfactory area. In the anterior end of the hemisphere it cannot be sharply separated from the lateral part of the area but farther caudad it is separated off by the fibers of the medial forebrain bundle. This nucleus as it is here described is practically the same as the medial parolfactory nucleus described for Cistudo carolina by Johnston ('15). Medialward it is bounded by the nucleus of the diagonal band of Broca.

Lateral parolfactory nucleus (figs. 7 to 9, 16, 17). The lateral parolfactory nucleus bulges out into the ventricle. Ventralward it follows the ventricle and cannot be sharply separated from the nucleus accumbens, so that by some writers the whole cell mass has been called nucleus accumbens septi. Dorsalward this lateral parolfactory nucleus, in the more anterior region of the forebrain, is clearly marked off from the primordium hippocampi by a cell-

free zone and, in the turtle, by the sulcus limitans hippocampi. Farther caudad, however, the line of separation between the two becomes indistinct. In the region just anterior to the commissure, if the writer has understood Johnston correctly, the sulcus disappears. Certainly in both the alligator and the turtle (Cistudo carolina) the cell-free zone disappears and there is no line of demarcation, so far as could be determined from the material available, between the primordium hippocampi and the more posterior portion of the nucleus parolfactorius lateralis. A cell mass is thus formed which has cells apparently of the type both of the primordium hippocampi and of the lateral parolfactory nucleus, although the latter type appears to predominate. Consequently, Herrick ('10) after a study of amphibian and reptilian material (including Lacerta, Cistudo carolina, and Alligator mississippiensis) and after an examination of embryonic reptilian brains from the Harvard collection, reached the conclusion that this nucleus was a part of his septal nucleus, consisting of cells of the basal region which had invaded this region, migrating upward along the descending hippocampal fibers.

Johnston ('13), on the other hand, has considered this intermediate cell group a part of the primordium hippocampi, basing his conclusion partly on the presence of the fornix fibers and the fibers of the hippocampal commissure, and especially on its position, as he believes from a study of embryonic material, above the neuroporic recess in a thickened portion of the lamina supra-neuroporica.

In the more anterior part of the brain, the ventro-lateral, small celled area (Johnston's caudate nucleus) is apparently continued around the corner of the ventricle to the medial surface (figs. 7 to 9, 16, 17). This continuation of the caudate has been termed nucleus accumbens by many observers. Johnston includes this nucleus accumbens in his nucleus parolfactorius lateralis.

It will be seen from the above discussion that the terms lateral septal nucleus (Herrick '10) and lateral parolfactory nucleus (Johnston '13 and '15) are not synonymous. If the writer has understood the authors correctly, the two masses compare

as follows. The lateral septal nucleus (Herrick '10) equals the lateral parolfactory nucleus (Johnston '13 and '15) plus a part of the posterior portion of the primordium hippocampi (Johnston '13 and '15) minus the nucleus accumbens (Herrick '10).

Having had no opportunity to study reptilian embryological material, the writer is in no position to decide which nomenclature is the better of the two. A most thorough study of the nuclei in the developing brain will be necessary before anything definite along that line can be determined. For convenience the terminology of Johnston has been adopted except that the name nucleus accumbens has been retained.

The essential point is that in the alligator, in the region of the fornix fibers and other descending hippocampal systems, there is a cell mass which serves as a place of synapse for many of the descending fibers. This cell group has been identified in a number of reptiles besides Alligator mississippiensis. Furthermore, so far as is now known, there are two possible sources of origin for this cell mass. The first theory is that it is a specialization of a portion of the primordium hippocampi as a place of synapse between the hippocampus and the basal centers. The second theory implies that cells, situated in the basal region and serving as places of synapse for descending fibers, moved upward toward their source of stimulation according to the principle of neurobiotaxis (Kappers, '14) and invaded the region of the primordiun hippocampi.

Tuberculum olfactorium (figs. 5, 6, 7, 15). This nucleus begins in the hemisphere a short distance behind the olfactory crus. It is ventro-medial in position and is continuous anteriorly with the nucleus olfactorius anterior, from which it can be distinguished by the cortex-like arrangement of its outer layer of cells. Its inner portion is made up of small groups of cells which show, though not so clearly as in some forms, the arrangement into islands so characteristic of the highly developed tuberculum olfactorium. The cortical and non-cortical layers of the tuberculum olfactorium are shown in the drawings of the toluidin blue sections from this region (figs. 5 to 7).

Laterally the tuberculum olfactorium lies in close relation to the pyriform lobe, although its cortical layer is separated from the latter by an area of scattered cells which belong apparently with the nucleus of the lateral olfactory tract (see discussion of that nucleus). Medially the tuberculum olfactorium is in close relation with the parolfactory region.

The relations described here are in all essential points the same as those described for the tuberculum olfactorium of turtles (Johnston, '15). The differences in the two forms are to be found in the somewhat higher development of the islands of Calleja in the turtle and in the absence in the turtle of so well developed a cortical layer as is found in the alligator. The tuberculum olfactorium does not appear as a clearly defined nucleus in Amphibia. In certain Dipnoi (Lepidosiren) described by Elliot Smith ('08) it appears in an exaggerated form. The Golgi material available does not show the cell forms in this region.

Nucleus commissurae hippocampi (figs. 18 and 19). This nucleus is really only a specialized portion of the primorium hippocampi, consisting of clusters of cells of that primordium which are mingled with the descending hippocampal fibers and which collect particularly about the point of decussation of the fibers of the hippocampal commissure. The cells of this nucleus serve as a place of synapse for commissural fibers and as cells of origin for some of the fibers of the medial cortico-habenular tract. In fact cells of this nucleus accompany this latter tract until it enters the stria medullaris and are probably a remnant of the broad gray connection found in lower forms between the hippocampal and the habenular regions.

Nucleus commissurae anterioris (fig. 18). This name has been given to the cells forming the bed nucleus of the anterior commissure. They resemble in general character the cells of nucleus preopticus but are quite distinct in type from those forming nucleus commissurae hippocampi. The cells of the nucleus commissurae anterioris afford a place of synapse for some of the fibers of the commissural division of the stria terminalis.

Nucleus preopticus (figs. 9, 10, 18 to 21). This term has been applied to the cell mass which appears in the region of the pre-optic recess just in front of the level of the commissures and which extends caudad still occupying this position. It passes over into the hypothalamic region with no definite line of separation between the two areas. The nucleus preopticus receives im-pulses from the stria terminalis, from fibers of the medial olfac-tory tract which have decussated by way of the anterior com-missure, and, at its anterior end, from some few fibers of the tract of the diagonal band of Broca.

Interstitial nucleus (figs. 10, 19, 20). Cajal ('11, vol. 2, p. 723) described this nucleus and figured it in the mouse, calling it "noyau interstiel de la voie de projection de l'écorce tempo-rale." He says further "Malheureusement, il ne nous a pas été possible de déterminer de façon précise les relations qui existent entre la bandelette semi-circulare et ce noyau, et cela à cause de la rareté des bonnes imprégnations. Ajoutans que cet amas de la région sousthalamique pourrait fort bien être encore un ganglion moteur."

Johnston ('15) has described the olfactory projection tract of Cajal for the turtle but has said nothing of the interstitial nucleus. In the alligator the nucleus appears in the preoptic region near the posterior end of the hemisphere as a ridge of cells extending lateralward in close relation with the nucleus ventro-medialis, arching dorso-medialward above the forebrain bundles and extending medialward into relation with the more dorsal part of the mass of the preoptic nucleus. It extends caudad throughout the preoptic region but in the hypothalamic region is gradually replaced by the hypothalamic nuclear ridge.

Part of the fibers of the olfactory projection tract of Cajal arise from the ventro-medial nucleus. Cajal ('11, vol. 2, p. 725, fig. 463) has shown some of the neurones of that nucleus giving rise to these fibers. Many of the fibers having such an origin quite probably send off collaterals among the cells of the interstitial nucleus. Part of the fibers of this olfactory projec-tion tract arises from the interstitial nucleus. The tract passes caudad with the fornix into the ventral part of the hypothalamus.

Nucleus of the diagonal band of Broca (figs. 8, 9, 17). This nucleus was first described for reptilian brains by Johnston ('15) in the turtle, Cistudo carolina. It is present in the alligator in practically the same relations as in the turtle. It appears behind the level of the tuberculum olfactorium as a dense collection of cells arranged in a cortex-like layer in the ventro-medial angle of the hemisphere. It extends dorsalward along the medial surface as a somewhat less dense, cortex-like layer which comes into relation with the medial parolfactory area and cannot be sharply distinguished dorsalward from the cell mass of the primordial hippocampus. It extends from the ventro-medial region lateralward, as scattered clusters of cells, into relationship with the nucleus of the lateral olfactory tract. The nucleus of the diagonal band extends posteriorly just outside of the medial forebrain bundle into the region of the preoptic nucleus. It is accompanied, as in the turtle, by bundles of fibers which serve for connecting the lateral and medial olfactory areas. The writer is particularly indebted to Dr. C. J. Herrick for aid in identifying this nucleus in the alligator.

Basal nuclei of the lateral wall. Students of the reptilian brain have generally recognized two basal centers in the lateral wall of the cerebral hemisphere, the corpus striatum and the epistriatum, and some have recognized a third region distinct from both of these, comparable with the mammalian nucleus amygdalae. According to these observers, the epistriatum is the more dorsal member of the complex and is in continuity with the cortical lamina. The extent of this continuity varies in different reptiles, depending upon the species and the general form relations of the hemisphere, particularly upon the ventro-lateral extent of the ventricle. In Testudo graeca, DeLange ('13a, p. 113, fig. 8.) has shown that the epistriatum is continuous with the lateral or pyriform lobe cortex throughout its whole extent. Kappers and DeLange consider the epistriatum to be striatal in origin and to have acquired secondarily a connection with the cortical lamina. They consider the epistriatum an olfactory nucleus of the second order and the entire epistriatum complex the homologue of the mammalian nucleus amygdalae.

On the other hand, Elliot Smith ('10) has considered the epistriatum to be of cortical origin.   He believes that the effecting of olfacto-somatic correlations in the reptilian hemisphere and, particularly, the entrance of tactual fibers into the dorsal part of the hemisphere lead to the disturbance of the morphological relations of the centers of the forebrain.   He says "One curious manifestation of these disturbing influences is seen in the ingrowth (toward the lateral ventricle) of part of the overgrowing pallium, forming a structure to which Edinger gave the name 'epistriatum.'   The epistriatum is not a part of the striate body but is cortical in nature.   Moreover it is not a morphological subdivision of the hemisphere which can be identified in other groups of vertebrates, as many anatomists believe.   It is merely a peculiar adaptation of structure to meet the conditions favorable to the reptile;—namely the disturbing influence of the recent admission of tactile impressions into the hemisphere."

In his 1915 paper, Johnston, following Edinger and Kappers (Kappers, '06, p. 9), suggests that the term 'epistriatum' be dropped, basing his suggestion on the facts that "the structure to which the term was first applied, does not appear as a special body or ridge in the turtle brain" and that "the author (Edinger) of the term uses it for at least three different bodies in the reptilian brain."   The conditions found in the forebrain of Alligator mississippiensis certainly support Johnston's suggestion.

In the anterior end of the hemisphere of the alligator several large cell masses are found in the basal portion of the lateral wall. 1.   There is a dorso-lateral area which includes a part or all of the 'epistriatum' as that term is used by some recent writers on the reptilian brain and is comparable with the dorsal ventricular ridge (Johnston, '15) in turtles, though perhaps not exactly homologous with that area.   2.   Below the dorso-lateral area, in a ventro-medial position, are two nuclei which belong to the corpus striatum of Johnston.   The more dorsal large celled mass is the ventro-lateral, large celled nucleus of this description, comparable with Johnston's nucleus lentiformis in the turtle. 3.   The more ventral small celled mass is the ventro-lateral,

small celled area of this description and is comparable with the area termed nucleus caudatus in turtles. 4. Between the dorso-lateral area and the ventro-lateral, large celled area, in the anterior end of the brain, there is an intermedio-lateral area which at first is closely tied up with the anterior part of the nucleus of the lateral olfactory tract, but later becomes continuous with the dorso-lateral area and probably is a part of that area. 5. The nucleus of the lateral olfactory tract is situated in the lateral part of the hemisphere, ventral to the dorso-lateral area and dorso-lateral and lateral to the ventro-lateral areas (the corpus striatum of Johnston's description). It lies in intimate relation with the intermedio-lateral area and is apparently continuous with it. Dorsalward it is at first clearly distinct from the dorso-lateral area but finally merges with it. Behind the level of the tuberculum olfactorium the more ventral part of the nucleus of the lateral olfactory tract becomes continuous with the nucleus of the diagonal band of Broca and then swings farther ventralward until it occupies the greater portion of the ventral region of the hemisphere internal to the cortex of the pyriform lobe and in close relationship with it. The nucleus of the lateral olfactory tract of the alligator, as the name is used in this paper, includes both the nucleus of that name and a small celled, ventral portion of the pyriform lobe as described in turtles. 6. In close relation with the nucleus of the lateral olfactory tract in the ventro-medial angle of the posterior half of the hemisphere is a nucleus to which the name of the ventro-medial nucleus has been given. This nucleus (figs. 18 to 21) gives rise to the projection tract of Cajal, and corresponds to the medial large celled nucleus described in turtles (Johnston, '15). 7. The outer ventro-lateral and ventral portions of this lateral wall are occupied farther cephalad by the cells of the tuberculum olfactorium and behind the level of that cell mass (8) by a part of the nucleus of the diagonal band of Broca. These two centers have been described previously. (For a more complete discussion of the extent, relations, and fiber connections of these nuclei of the lateral wall see the special headings.)

Dorso-lateral area (figs. 7 to 10, 12, 15 to 19). This area forms most of the large eminence which projects from the ventro-lateral wall of the hemisphere into the lateral ventricle and nearly fills that cavity. Its lateral aspect is exposed in the dissection illustrated in figure 2. The anterior end of the dorsal area is marked by the previously mentioned inward fold of the dorso-lateral cortex (figs. 5, 6, 7) which may be considered primordial general pallium. In the turtle, as many writers have shown, there is a pallium-like infolding throughout the whole extent of a somewhat similar area, the dorsal ventricular ridge of Johnston ('15). In all but its most anterior portion, the dorsolateral area in the alligator is cut off from the pallial areas by the outward and downward growth of the lateral ventricle, so that the infolding can be plainly seen only in the anterior end of the hemisphere. The dorso-lateral area is bounded ventrally by the intermedio-lateral area and then by the ventrolateral large celled area and ventro-laterally by the anterior end of the nucleus of the lateral olfactory tract (figs. 7, 8). Behind the ventro-lateral areas (the lentiform and caudate nuclei of Johnston) it is bounded ventrally by the posterior portion of the nucleus of the lateral olfactory tract (fig. 12). Olfactory fibers from the lateral olfactory tract distribute to the dorsolateral area from behind the level of the general cortex infolding to the posterior end of the area. Ascending somatic sensory fibers from the thalamus also distribute throughout practically all parts of this region and, in some parts of the area, these somatic connections alone are present without admixture with olfactory fibers. This purely somatic region includes practically all the dorso-lateral area at the anterior end of the brain. Farther caudad an increasingly large amount of the lateral and dorso-lateral portions of this area receives olfactory fibers and only the dorso-medial portion is relatively pure somatic in type of correlation. The ridge of primordial general cortex implies a very close relation between the somatic dorso-lateral area and the general cortex.

The ventro-lateral areas as they have been termed in this description of the alligator brain are apparently directly compara-

ble with the corpus striatum which Johnston has described in turtles. If that author has been correctly interpretated, the ventro-lateral small celled nucleus of this description is nucleus caudatus, while the ventro-lateral large celled nucleus is the nucleus lentiformis of turtles. The writer has avoided the specific terms employed by Johnston because she does not have sufficient knowledge of the development of the striatum throughout the vertebrate series to be certain of the homologies.

Ventro-lateral small celled area (Johnston's nucleus caudatus). This nucleus (figs 7 to 9) begins a short distance behind the olfactory crus and, increasing in size, extends backward to the level of the anterior nucleus of the thalamus with which its posterio-medial portion lies in close relation. Posteriorly its ventral and lateral portions lie in close relation with the posterior part of the nucleus of the lateral olfactory tract. Anteriorly it cannot be sharply delimited from the nucleus olfactorius anterior. Johnston ('15) in turtles, where the relationships of the caudate nucleus are practically the same as the relationships of this area in the alligator, has considered the nucleus olfactorius anterior and some associated gray as giving rise to the head of the caudate nucleus in mammals. The great increase in the neopallial area in higher forms is accompanied by an increase in the number of fibers (internal capsule fibers) distributing to that cortical area. These fibers are imbedded in the caudate nucleus and more posteriorly are ventral to it and so push this cell mass dorsalward and caudalward as they increase in number during phylogeny. The upward, backward and downward growth of the general cortex and the downward growth of the pyriform lobe have produced the typical curve of the caudate nucleus of higher forms.

The ventro-lateral small celled area is continuous around the ventral border of the ventricle and onto its medial surface and this continuation represents the nucleus accumbens of higher forms. This apparently belongs to the striatum complex, although Johnston ('13, p. 421) has joined it to the nucleus lateralis septi of previous authors under the name of nucleus parolfactorius lateralis (see the discussion of the parolfactory nuclei).

Ventro-lateral large celled area (Johnston's nucleus lentiformis) (figs. 7 to 10, 17 to 19). This nucleus appears as a group of cells just dorsal to the small celled area at the anterior of the hemisphere and laterally close to the nucleus of the lateral olfactory tract. Farther caudad it is partially separated from the ventro-lateral small celled area (Johnston's caudate) by a special fascicle of the lateral forebrain bundle. In the posterior part it forms a heavy ridge of cells over the dorsal and dorsolateral portions of the small celled area. It disappears in front of the posterior end of the latter area at about the level of the foramen of Monro. The larger size of the cells of the ventrolateral large celled area makes it easy to distinguish this nucleus from the ventro-lateral small celled area.

Intermedio-lateral area (figs. 7, 8). This area is found at the level of the posterior part of the infolding of the general cortex and in the region just caudad to that infolding. The intermedio-lateral area is ventral to the dorso-lateral area, dorsal to the ventro-lateral areas and medial to the nucleus of the lateral olfactory tract. It lies in so close relation with this last nucleus especially in its more anterior extent that it is not practicable to attempt to draw any sharp boundary line between them. The intermedio-lateral area is separated from the ventro-lateral areas by a cell free zone in which are fibers which belong in part at least to the lateral forebrain system. A sulcus in the ventricular wall indicates the position of the boundary line between the intermedio-lateral and ventro-lateral area. The anterior portion of the former area is separated from the dorso-lateral area by a cell free zone but the two areas fuse into one, behind the level of the infolding of the general cortex. So far as the evidence goes, the intermedio-lateral area appears to belong with the dorso-lateral area. Possibly it is a representative of some part of the striatum complex of higher forms.

Nucleus of the lateral olfactory tract (figs. 5 to 10, 12, 16 to 19). This nucleus can be distinguished from the other cell masses in the lateral wall of the hemisphere by the smallness of its cells. It begins as a small cluster of cells scattered along the inner border of the cortex of the pyriform lobe and between that cor-

tex and the cortex-like superficial layer of the tuberculum ol-
factorium.   Close to its anterior end the cells of the upper or
more dorsal portion of the nucleus group themselves more close-
ly together and a clearly defined nucleus is formed which is ven-
tral to the dorsal-lateral area and lateral to the ventro-lateral
large celled area.   At first this upper portion of the nucleus of
the lateral olfactory tract remains distinct from the surrounding
cell masses of the hemisphere wall; but as it is followed caudad
it gradually comes into close relation with the cell mass of the
dorso-lateral area and finally merges with it with no sharp de-
limiting line between the two, a greater and greater number of
large cells appearing among the small cells in that region until
apparently the mass has become a part of the dorso-lateral area.
The ventral portion of the nucleus, at the anterior end of the
brain, consists of diffuse clusters of cells lying in close relation
with the pyriform lobe, the cortex of the tuberculum olfactorium,
and, farther caudad, the nucleus of the diagonal band of Broca.
At approximately the level of the fusion of the anterior dorsal
portion of the nucleus of the lateral olfactory tract with the
dorso-lateral area, the ventral portion of the former nucleus
broadens out and extends to the posterior end of the hemisphere,
occupying first a ventro-lateral and then a ventral position.

A part (probably the more ventral portion) of the anterior
dorsal portion of this nucleus of the lateral olfactory tract, as it
has been described for the alligator, is quite probably compar-
able with the small celled, ventral part of the pyriform lobe
observed by Johnston ('15) in the turtle.   In describing this small
celled part Johnston says that in the rostral part of the brain of
Cistudo carolina it may be sharply distinguished from the large-
celled portion both by the difference in cell character between
the two regions and by the more ventral position of the small
celled portion, which extends below the sulcus endorhinalis and
expands behind the posterior part of the striatal area into
the nucleus of the lateral olfactory tract.   In the alligator the
continuance of the pyriform lobe cortex (Johnston's large celled
medial portion) farther ventralward, has pushed this small celled
portion inward and crowded it somewhat dorsalward so that

it lies for the most part, medial to the pyriform lobe cortex instead of ventral to it as in turtles. Furthermore the anterior, dorsal end of the nucleus of the lateral olfactory tract (Johnston's small celled, ventral portion of the pyriform lobe) is much larger in the alligator than in the turtle and this increase in size has probably been another important factor in bringing about the change in the relative positions of the two cell masses.

The more ventral and posterior portions of the nucleus of the lateral olfactory tract as that nucleus has been described for the alligator are comparable to nearly all of the nucleus of that name described for the turtle (Johnston, '15). Here again, however, there is one point of difference, for, while in the turtle the nucleus occupies the outer portion of the hemisphere in the posterior part of the forebrain, in the alligator the cortex of the pyriform lobe extends downward and occupies the outer portion of the ventral wall, the nucleus of the olfactory tract lying internal to it and in close relation with it. (For a further discussion of these relations and their significance see the account of the pyriform lobe.)

To summarize, the nucleus of the lateral olfactory tract as it is present in the alligator is practically the equivalent of the nucleus of that name and the small celled ventral part of the pyriform lobe in turtles, except that the last named area has been greatly elaborated in the alligator and its more anterio-dorsal portion may very well have taken on an added significance from its intimate relation with the somatic dorso-lateral, area.

Ventro-medial nucleus (figs. 10, 12, 18, to 21). This nucleus occupies the extreme ventro-medial portion of the hemisphere, extending throughout about the posterior half of the hemisphere. It has broad connections with the habenula by way of the stria medullaris and it gives rise to the great olfactory projection tract of Cajal.

This quite evidently is the medial, large-celled nucleus described by Johnston ('15) for Cistudo carolina, although the cells of this nucleus, in the alligator, resemble in size and in cell characteristics the cells of the nucleus of the lateral olfactory tract, except that they are massed somewhat more closely together.

Functional complexes formed by the basal nuclei of the lateral wall. In the foregoing paragraphs, an account has been given of the relative positions and the extents of the various nuclei found in the lateral wall of the hemisphere, and something has been said of their fiber connections. Two problems then arise, the first regarding the way in which these centers interact in the functioning brain of the alligator; the second regarding their phylogenetic significance as forerunners of centers found in mammalian brains.

Two types of nervous impulses enter the lateral wall of the cerebral hemisphere, (1) descending impulses from the olfactory area, and (2) ascending somatic sensory impulses from the centers of the thalamus. The nuclear pattern of this basal area of the forebrain has been determined in large measure by the distribution and mutual interconnections of the incoming fibers of these two systems.

The first type of nervous impulse includes the secondary and tertiary fibers of the lateral olfactory tract, which, entering from in front, distribute to the nucleus of that tract throughout its entire extent and, turning gradually dorsalward, in the posterior half of the hemisphere distribute to the lateral portions of the dorso-lateral area. The lateral part of this dorso-lateral area, the nucleus of the lateral olfactory tract, and the ventro-medial nucleus all give rise to fibers of the stria medullaris and the first two masses (and in turtles the ventro-medial nucleus also) discharge through the stria terminalis. The ventro-medial nucleus in both the turtle and the alligator discharges into the diencephalon through the great olfactory projection tract of Cajal.

The identification of the amygdaloid complex of higher forms is based on the following features: 1) upon its reception of fibers from the lateral olfactory tract (figs. 16, 18); 2) upon its relation to the pyriform lobe cortex (figs. 7 to 10); 3) upon its giving rise to fibers of the stria terminalis (figs. 19 to 21—in this is included its connection with the opposite side of the brain by way of the anterior commissure); 4) upon its giving rise to fibers of the stria medullaris (figs. 16 to 21). It is evident that the group of centers

just discussed, i.e., the nucleus of the lateral olfactory tract, the ventro-medial nucleus, and the more lateral part of the dorso-lateral area, make up such an amygdaloid complex.

The second type of impulse which enters the lateral wall of the hemisphere is somatic, being transmitted by the somatic sensory radiations from the lateral and medial nuclei of the thalamus to the ventro-lateral areas (caudate and lentiform nuclei). These areas, then, are centers for the correlation of somatic sensory impulses in the hemisphere and are, therefore, the forerunners of the mammalian corpus striatum. They discharge into the lower brain centers through the lateral forebrain bundle.

A part of the somatic sensory fibers pass beyond the ventro-lateral large celled area (Johnston's nucleus lentiformis) into the dorsal area, so that at the level of the primordial general cortex (figs. 5, 6, 15), this dorsal area is entered almost exclusively by the somatic correlation fibers and hence is a somatic correlation center of striatal type. This area at its anterior end probably exhibits the highest type of somatic correlation tissue found in the brain of the alligator, and the entrance of association fibers from the adjacent cortical centers into its dorsal part has given the conditions favorable for the differentiation of primordial general cortex (i.e., cortex approaching the neopallial in type).

It will be remembered that behind the level of this thickening representing primordial or transitional general cortex the lateral part of the dorso-lateral area receives olfactory fibers and probably some somatic fibers, and belongs to the amygdaloid complex. Whether this portion becomes a somatic part of the amygdaloid complex of higher forms, as Johnston ('15) believes is the case with the dorsal ventricular ridge in turtles, or whether it is a step toward the enormous striatum complex found in birds, cannot be decided without a much greater knowledge of other vertebrate forms than the writer possesses. In any case, it is very evident that the dorso-lateral area must be regarded as a structure of intermediate or transitional type, containing primordia related to three diverse structures in the mammalian brain, viz., corpus striatum, amygdaloid complex, and the general cortex.

Cortical centers of the hemisphere. Within the pallium three types of cortical centers may be distinguished. One of these, the hippocampus, is concerned primarily with olfacto-visceral correlations. The pyriform lobe cortex is concerned chiefly with olfacto-somatic correlations, with some involvement of the general visceral centers of the hypothalamus. The third type, the general cortex, is largely concerned with somatic correlations, and is differentiating toward true neopallium.

Hippocampus (figs. 3 to 10, 12, 14 to 21). Spitzka was the first to suggest that the dorso-medial wall of the hemisphere was hippocampus, although he still called the hippocampal commissure the corpus callosum. From that time the homology of this medial cortex has been recognized by most observers, including Edinger, Brill, Meyer and Elliot Smith. Very good summaries of the earlier studies on the hippocampus and its commissure are given in Elliot Smith's article ('03) dealing with the morphology of the cerebral commissures in vertebrates and in the Arris and Gale lectures ('10). In this connection it is interesting to note that Johnston ('15) has reopened the question of the presence of true callosal fibers in the dorsal or hippocampal commissure of both marsupials and reptiles. His evidence for their presence is based on experimental work. In regard to reptiles he says, page 404, "in the turtles the lack of medullation in the dorsal commissure has made it impossible thus far to secure positive evidence as to the presence of callosal fibers." He argues that they should be present because of the great number of ascending fibers carrying sensory impulses from the thalamus to the telencephalon in reptiles. Others, as Ramón y Cajal, Unger, and Pedro Ramon, have claimed that various reptiles have true callosal fibers.

Adolf Meyer ('92) was the first person to distinguish between the dorsal and the dorso-medial portions of the hippocampus. The dorso-medial portion arises rostrad in the narrow part of the olfactory crus and there occupies a somewhat dorsal as well as a dorso-medial position (fig. 3). At this level it lies in close relation dorso-lateralward with the cortex of the pyriform lobe. As it extends caudad into the hemisphere, the dorso-medial

cortex takes its characteristic position and dorsal to it appears a group of scattered cells of a larger size which, judging from their fiber connections, are strongly under the influence of the dorso-medial portion. This latter group constitutes Adolf Meyer's dorsal portion of the hippocampus and is the 'subiculum' described by Johnston ('15) in turtles. Except at the very anterior end of the hemisphere, the hippocampal formation and the cortex of the pyriform lobe are separated by the general pallium. Ventralward, the dorso-medial portion of the hippocampus is continuous with a diffuse mass of small cells, the primordium hippocampi.

In the more anterior part of this dorso-medial region of the hippocampus, the cells, as seen in Golgi preparations, are goblet-shaped and are comparable with the cells of the secondary olfactory nuclei. They are more nearly related in type to the small projection cells of the hippocampus. The most anterior portion of the hippocampus probably does function to considerable degree at least, as a secondary olfactory nucleus. Other cell types found in the hippocampus are as follows:

1. Correlation cells (fig. 31). The correlation cells of this type are found in the dorsal portion of the dorso-medial area at the anterior part only, so far as is known. They are especially interesting because of their resemblance to the cells of the hippocampal regions in Amphibia (Herrick, '10). These are probably phylogenetically the oldest of the highly specialized cells of the hippocampus.

2. Double pyramid cells (figs. 35, 36). These are the cells which especially give character to the dorso-medial portion of the hippocampal cortex. Their cell bodies are large and more or less pyramidal in form. Thick, thorny, bushy dendrites spread out lateralward and medialward from the cell body but are especially thick on the medial side, where they can often be seen breaking up around the terminal arborizations of the incoming medial olfactory, parolfacto-cortical and tuberculo-cortical tracts. Sometimes the impulse reaches the double pyramid cell through an interpolated neurone. The dendrites which are directed lateralward, receive olfactory impulses from the

pyriform lobe and the nucleus of the lateral olfactory tract. These impulses come by way of the alveus which carries impulses in both directions, as it does in higher forms. The laterally directed dendrites receive short association fibers from the alveus and perhaps impulses from other incoming fibers.

The axones of the double pyramids are slender and run lateralward, dividing in many cases into two branches (fig. 36). One of these branches enters the alveus and can often be traced a long distance, although it has been impossible as yet to follow any single fiber all the way into the pyriform lobe. The second branch, when present, goes to the septum or enters one of the descending diencephalic tracts (the fornix or tractus cortico-habenularis).

3. Small projection cells (figs. 32, 33). Besides the double pyramid cells there are other projection cells in the hippocampus. These are smaller than the ones just described and may be either pyramidal, oval or nearly round in form. They are usually either slightly lateral or slightly medial to the double pyramids and send their dendrites to both lateral and medial surfaces, where they receive the same sorts of impulses as are brought to the double pyramids. The axones, like those of the latter, may divide into two branches (fig. 33), one entering the alveus and the other running ventralward into the septum and presumably, in some cases, entering tracts descending to the diencephalon.

4. Small intrinsic cells (fig. 34). The dendrites of the double pyramid and small projection neurones form a thick feltwork on each side of the more deeply placed cell bodies. Scattered through this feltwork are cells of several types, only one type of which is shown in the figures, which send out relatively short bushy dendrites and receive collaterals from incoming fibers or from axones of the hippocampal projection cells and discharge back into the dendrites of the latter. In this way the whole of the hippocampus is tied up together and correlated and unified responses are made possible. Some of these cells are typical type II neurones, others have longer, less branched processes and short slender axones. Both of these sorts are apparently intrinsic to the hippocampus.

Levi ('04) has described cells of the double pyramid and small projection types in the hippocampus of reptiles. The account given here agrees substantially with the descriptions and figures given in his article. It is interesting to note that the medial side of the dorso-medial cortex, as Levi suggests, appears to be concerned mainly with the reception of incoming stimuli from the lower brain centers, while, on the other hand, the main projection fibers which connect the hippocampus with the pyriform lobe (the alveus) and with the diencephalon (fornix and the tractus cortico-habenularis medialis) leave on the lateral side. In the turtle the hippocampal cortex lies close to the ventricle. Presumably, in that case, many of the efferent fibers leave on the medial side, but so far as is known, there is no literature on that subject. It is certain, however, that the dorso-medial region of the hippocampal cortex of the alligator represents a higher differentiation than the corresponding region in the brain of the turtle and that this differentiation is marked, not only by a more definite cell arrangement and possibly by a more specialized cell form, but also by a new position of the cell mass produced by a biotactic movement of the cell group away from the ventricular wall and in the direction of the incoming impulse.

Meyer ('92) and Levi ('04) claimed that the dorso-medial portion of the reptilian hippocampus was gyrus dentatus. This contention was denied by Ramón y Cajal, who, in an elaborate series of histological studies, showed that from its cell types and manner of connection, it could not be considered pure gyrus dentatus. In the Arris and Gale lectures ('10), Elliot Smith admits the correctness of the Cajal observations but says that the dorso-medial portion is undergoing a differentiation toward the production of gyrus dentatus and that it is the forerunner of that structure. This seems a fair statement of the conditions.

The dorsal portion of the hippocampal cortex does not show the regular arrangement of cell layers found in the dorso-medial portion, and in general, its mass of cells shows a lighter staining reaction to toluidin blue. Figure 31 shows one type of correlation cell found in this dorsal region. This dorsal part appears to be concerned chiefly with the association of impulses rather

than as a receptive center, its main incoming impulses, so far as known, coming in through the alveus. As Elliot Smith and Levi have suggested, this is probably the forerunner of the hippocampal cortex as distinguished from the gyrus dentatus. Johnston ('15), however, regards this dorsal cortex as the forerunner of the mammalian subiculum. From the region of the infolding of the primordial general cortex (fig. 6) in the alligator this dorsal part of the hippocampal cortex is continuous with the general cortex.

The presence in the alligator of a primordium hippocampi, such as Johnston ('13 and '15) has described in turtles, has already been mentioned. In the turtle that author has shown the presence, on the medial wall of a fimbrio-dentate sulcus (Elliot Smith's sulcus limitans hippocampi) between the dorso-medial portion of the hippocampus and the primordium hippocampi and a sulcus limitans hippocampi and a cell free zone between the primordium hippocampi and the parolfactory area (Herrick's ('10) septal area) in the anterior part of the brain. On the ventricular side of the medial wall in the turtle are two sulci which correspond to those on the lateral wall and separate the same areas. In the alligator in the material which was studied, no well defined sulci are in evidence on the medial surface of the hemisphere in these regions but the ventricular sulci are present in positions corresponding to those in which they are found in the turtle; and the primordium hippocampi, in the more anterior part of the hemisphere, is separated from the septal or parolfactory area by a cell free zone. An interesting fact, but one whose significance is not clearly understood, is the presence on the ventricular surface of a relatively thin ependymal layer over the dorso-medial portion of the hippocampus and of the primordium hippocampi, which becomes thickened over the septal or parolfactory region. Under the head of the parolfactory area, the relative positions and the relations of the primordium hippocampi and the lateral parolfactory nucleus have been discussed.

Johnston ('13, figs. 23 to 27, pp. 446–447) has shown that the primordium hippocampi extends forward in the hemisphere considerably anterior to the hippocampus proper. In the alligator

the dorso-medial cortical area extends forward into the region just posterior to the olfactory crus and the primordium hippocampi, though quite plainly present, is relatively smaller than in the turtle. This means that in the alligator the hippocampus in this region is more highly specialized than in the turtle. The cells making up the primordium hippocampi are small and are arranged in a diffuse mass. They are not impregnated in the Golgi preparations which were studied.

Pyriform lobe (figs. 3 to 10, 12, 14 to 19). The pyriform obe has important functions both as a secondary olfactory center and as a correlation mechanism of high order. By means of connections with the olfactory bulb and the basal and cortical centers of the hemisphere it receives both correlated and uncorrelated olfactory material. By means of its connection with the tuberculum olfactorium and, also, through short correlation fibers from the somatic centers of the hemisphere, t receives correlated somatic material. It receives impulses from the other cortical centers by way of the alveus. Consequently it serves, in part at least, as an olfacto-somatic correlation center of high order.

In the anterior end of the hemisphere, in the region of the olfactory lobe, the pyriform lobe cortex and the hippocampal cortex lie in close relation with each other dorsally. They are soon separated, however, by the general pallium which intervenes between them throughout the remaining extent of the hemisphere. Ventrally the pyriform lobe lies in close relation with the tuberculum olfactorium, separated from it only by some scattered cells of the anterior division of the nucleus of the lateral olfactory tract. Behind the tuberculum olfactorium, the cortex of the pyriform lobe is bounded ventrally by the nucleus of the diagonal band of Broca and by the nucleus of the lateral olfactory tract. Near the posterior end of the hemisphere, this latter nucleus, which there occupies all the ventral surface excepting the portion occupied by the ventro-medial nucleus, acquires a cortex-like arrangement of its superficial cells (fig. 12) which layer is continuous with the cortex of the pyriform lobe and to all intents and purposes is a part of that area since the pyriform

lobe cortex itself arose as a differentiation of the neurones of the nucleus of the lateral olfactory tract (see Johnston '15 and the general discussion at the end of this paper).

Medialward the pyriform lobe cortex is bounded by the dorso-lateral area and by the anterior part of the nucleus of the lateral olfactory tract.   This anterior division bears about the same relation to the pyriform lobe cortex that the primordial hippocampus bears to the hippocampal cortex proper, i.e., it consists of cells which have practically the same type of connections as do the cells of the pyriform lobe cortex.   It represents the general area from which the specialized pyriform lobe cortex has developed.   In this paper it has been considered as the anterior part of the complex of the nucleus of the lateral olfactory tract, but it might equally as well be termed primordial pyriform lobe cortex or the small celled portion of the pyriform lobe complex, as Johnston ('15) has called it in turtles.   (For a further description of this anterior division of the nucleus of the lateral olfactory tract see the description of that nucleus).

The writer has not been able to identify the sulcus endorhinalis and the sulcus rhinalis is slight but does show in some preparations.   The cell type illustrated in figure 39 is found in the anterior end of the pyriform lobe.   It resembles the cells found in the secondary olfactory centers, which is not surprising since the anterior end of the pyriform lobe cortex itself probably serves to a considerable degree as such a secondary center. The Golgi material available for study does not show the cell types found in the more posterior part of this cortex.

General cortex (figs. 4 to 10, 12, 16 to 19).   As has been stated, the cortex of the pyriform lobe and the hippocampal cortex are separated from each other by the general cortex except at the anterior end of the brain in the region of the olfactory lobe. In many reptiles this cortex forms a definite lamina separated from the other cortical areas by distinct limiting zones but in the alligator, at least in the material studied, no such sharp limiting zones are visible.   Medialward as in the turtle, it grades over into the thicker dorso-medial part of the hippocampal cortex (Johnston's subiculum, '15).   Lateralward it is continuous with

the cortex of the pyriform lobe. The rhinal fissure is demonstrable in some of the material but is relatively slight.

In the anterior end of the dorso-lateral area of the hemisphere there is a ridge of cortex-like cells which has the appearance of being a fold of the general cortex. This has been termed in the present paper, primordial general cortex. Between the primordial portion and the general cortex proper, at the medial border, there is a small space (fig. 6) which permits association fibers of the alveus system to reach the former portion. Johnston ('15) has described in turtles a ridge of cells similar to the primordial general cortex of this description. He calls it the dorsal ventricular ridge but says that it belongs to the general pallial complex (the general cortex of this description). In a later paper ('16) he shows that both the ridge and the general cortex are derived during embryonic development from the dorso-lateral area. Phylogenetically the general cortex complex arose under the influence of at least two types of fibers. 1. The one type consists of fibers carrying impulses from the somatic centers of the diencephalon to the dorso-lateral area of the forebrain by way of the lateral forebrain bundle. 2. Into this dorso-lateral area association fibers from the hippocampal and pyriform lobe complexes also distribute. As these latter areas differentiated a higher type of integrated impulses was brought in and, within the dorso-lateral area, neurones exhibiting a cortex-like type of differentiation appear. In this way within the basal dorso-lateral area, a primordial general cortex is probably formed. What the factors were which caused this primordial cortex to become more superficial in position and to separate from the basal dorso-lateral area to take on a true cortical form, of course is not certainly known. Perhaps one cause lies in the neurobiotatic influence of the association fibers, the neurones migrating out along their dendrites toward the source of their stimulation.

The whole of the general cortex complex is a step toward the differentiation of a neopallial area. To be sure, this complex is still closely linked with cortical olfactory areas, but it has a relatively large somatic component and its connection with the basal somatic areas is intimate. As maintained by Elliot Smith and

others, it cannot be regarded as true neopallium, but rather represents a process of differentiation in that direction.

Johnston ('16a) by a series of experiments on the turtle brain has reached the conclusion that there is some degree of cortical localization in the general pallium of that reptile. The writer at present has not sufficient data to determine whether or not there is any localization pattern in Alligator mississippiensis.

Centers of the diencephalon

The diencephalon may be divided into the three usual divisions (1) the epithalamus; (2) the thalmus; (3) the hypothalamus. It is not the purpose of this report to go into the question of nuclear localization in these regions nor to attempt to describe the character of the cell groups. There has not been sufficient work done to justify such an attempt. Only a few of the more outstanding facts of especial interest in connection with the discussion of the forebrain will be mentioned.

At the end of his 1913 paper, DeLange has given a series of outlines of the diencephalon of the alligator in which the positions of the various nuclei and their topographic relations to the various fiber tracts are indicated. These have been of the greatest help.

Epithalamus. The part of the epithalamus which is particularly concerned with the reception of olfactory impulses is the habenula (figs. 11, 12, 21). This nucleus lies at the dorsal surface of the diencephalon and projects into the ventricle. The stria medullaris brings impulses to this nucleus. It consists of three smaller nuclei; a medial one of closely packed cells, a dorsal and more anterior one which apparently receives part of the tractus cortico-habenularis medialis and, lastly, a ventral one of larger cells that, farther caudad, connects with the cell mass of nucleus magnocellularis. The habenulae of the two sides connect with each other by means of the commissura habenularum (fig. 12).

Thalamus. There are really three types of nuclei in the thalamus proper: a medial group which connects chiefly with the

visceral centers, a lateral group which is the place of termina-
tion for the somatic impulses brought in by the optic and lem-
niscus systems and, intermediate between these two groups, a
third nucleus which receives fibers of both the visceral and
somatic type. This nucleus is the nucleus medialis or the
nucleus rotundus of some authors.

In the medial group are the nucleus anterior and the nucleus
magnocellularis. The nucleus anterior (figs. 10 and 11, 20), as
its name implies, lies at the very anterior end of the thalamus.
It is dorsal in position and its cells are smaller and more closely
packed together than are the cells of the lateral nucleus. It re-
ceives fibers from the hypothalamus and is connected with the
small celled ventro-medial part of the hemisphere by means of
a fiber tract.

The lateral group includes the nucleus lateralis, a special
derivative of this nucleus—the pulvinar—and another optic
center which most writers have termed the corpus geniculatum
laterale. The nucleus lateralis is conspicuous because of the
large size of its neurones. The cell bodies of these neurones
(figs. 41, 42, 43) are large, goblet or triangular in shape, and have
thick thorny dendrites which extend out in every direction from
the cell bodies. The axones enter the lateral forebrain bundle.
This nucleus is lateral in position, being lateral and somewhat
ventro-lateral to the nucleus anterior and lateral to the nucleus
medialis (or rotundus). It receives lemniscus fibers and some
optic fibers and, with the lateral thalamic optic centers, repre-
sents the beginning of the neothalamus (Edinger) of higher
forms, i.e., that lateral portion of the thalamus which serves
as a place of synapse for nervous impulses passing to the neo-
pallium and which develops parallel with the development of
the neopallial cortex. In the more posterior part of the thala-
mus, a lateral portion has begun to differentiate away from this
nucleus and to form a beginning of the pulvinar. This separate
nucleus is developed under the direct influence of the incoming
optic fibers.

There are other cell masses in the thalamus proper, as for
example the nucleus reuniens figured in the alligator brain by

DeLange ('13); but the writer knows too little of their relationships or significance at present to discuss them.

Hypothalamus. The hypothalamus of the alligator is highly developed. An examination of figures 11 and 12 will show that a number of cell groups are present. In his 1913 paper DeLange has named these different groups. No attempt has been made to do so in the present paper because of a lack of knowledge of the fiber connections of the different groups.

FIBER CONNECTIONS

With the foregoing descriptions of the cell groups as a basis, attention can now be turned to the courses and terminations of such of the fiber tracts as have been worked out. Papers published by C. L. Herrick, Edinger, Adolf Meyer, Kappers, Unger, DeLange, and Johnston contain descriptions of the fiber connections of the reptilian brain. These descriptions in almost every case, have been based on adult material, the work being done with Weigert preparations which bring out the myelin sheaths. On the other hand, the work for this paper has been done chiefly with Cajal and Golgi material, which bring out the unmyelinated fibers and, in many cases, the axis cylinders of the myelinated ones. Repeated attempts to prepare a series stained by the Weigert method were not successful so far as the forebrain was concerned. These failures, of course, may have been due to faulty technique, but only extremely young material was available and in such material many of the myelin sheaths may not have become mature. C. J. Herrick ('10) has figured on pages 537, 539, and 541 some cross sections of the forebrain and the thalamus of Alligator mississippiensis showing the fiber tracts and the positions of some of the centers. These drawings were made from Cajal material and were of much help. A series stained with Ehrlich's haematoxylin and an imperfect series prepared by the Leuden van Heumen method were used to check the results obtained by the Cajal method.

Tractus olfactorius

Following the human terminology, the writer has considered this tract to consist of three divisions, a medial, an intermediate, and a lateral, although the first two are very closely associated and have both been considered by many authors under the name of the medial olfactory tract. (For a diagram of the distribution of these tracts see figs. 44 to 46). The data given here have been obtained, partly by the study of sections prepared by the use of Ehrlich's haematoxylin and by the Leuden van Heumen method and partly by work with a Cajal series in which the axis cylinders of any myelinated fibers, as well as the unmyelinated fibers, were brought out. The data in all probability are not complete.

Tractus olfactorius medialis (figs. 13, 14, 44). This tract has been described in reptiles by Edinger ('88), Adolf Meyer ('92), C. L. Herrick ('93), Unger ('06), DeLange ('11), and Johnston ('15). As its name implies, it lies medial to the ventricle of the bulb and arises, in general, from the more medially and ventromedially placed mitral and granule cells. Throughout the bulb, this tract is lateral to the mitral cells. In the crus many of its fibers end in the nucleus olfactorius anterior or send their collaterals to that nucleus. The projection cells of the nucleus, in turn, send axones to join the tract. Thus the medial olfactory tract is made up of fibers from both primary and secondary olfactory centers.

In the anterior end of the hemisphere the tractus olfactorius medialis has come to lie along the medial surface and it occupies this medial position as it passes caudad, discharging at various levels into the dendrites of the intrinsic cells, the double pyramids and the small projection cells of the hippocampus.

Tractus olfactorius intermedius (fig. 14). This fiber tract, arising from mitral and granule cells and not distinguishable from the medial tract until the hemisphere is reached, ends in the nucleus olfactorius anterior and the medial part of the tuberculum olfactorium. Other fibers pass farther caudad and appear to enter the nucleus of the diagonal band of Brocá. It is

joined by fibers from the nucleus olfactorius anterior to the tuberculum olfactorium. Part of its fibers pass through the anterior commissure to the other side and end there in the nucleus olfactorius anterior and, probably, partly in the tuberculum olfactorium. These connections have been described by nearly all the later workers on the reptilian brain.

Johnston ('15) in the turtle has considered the medial and intermediate tracts both under the name of the medial olfactory tract. He describes a very interesting bundle of this medial tract which runs caudad with the fiber bundle of the diagonal band of Broca to the nucleus of the lateral olfactory tract. Quite probably this tract is present in the alligator but the material available does not permit of its identification.

Tractus olfactorius lateralis (figs. 13 to 19, 44 to 46). Edinger ('88), Adolf Meyer ('92), C. L. Herrick ('93), Unger ('06), Kappers and Theunissen ('08), DeLange ('11), and Johnston ('15) have described this tract in reptiles. In general it arises from the more laterally placed mitral cells and projection granule cells of the bulb but some of the fibers come from the dorso-medial portion and cross over to join the lateral tract. Like the medial tract, this lateral one at first lies internal to the mitral cell layer. While still in the bulb it begins to swing out to the surface and in the crus and lobe it lies mainly along the lateral border of the hemisphere. Near the anterior end of the hemisphere it divides into an outer and an inner division. The inner division enters the nucleus of the lateral olfactory tract and distributes to it throughout its whole extent. The outer division ends in synaptic relations with cells of the pyriform lobe and sends some fibers to the more lateral portions of the tuberculum olfactorium. The tractus olfactorius lateralis not only sends fibers to the pyriform lobe but, in the crus and the anterior end of the hemisphere, at least, it also receives fibers from it. This tract, then, carries both secondary and tertiary impulses.

Tractus tuberculo-corticalis

From the tuberculum olfactorium a band of fibers runs along the medial border of the hippocampal cortex and discharges

into the dendrites of the hippocampal cells. This is the tractus tuberculo-corticalis (figs. 14, 15). Some fibers swing to the lateral side of the hippocampus; these may be cortico-tubercular fibers. Farther cephalad some olfacto-cortical fibers from the nucleus olfactorius anterior join this tract.

Parolfacto-cortical tracts

Tractus parolfacto-corticalis (figs. 16. 17). Fibers swing upward from the medial parolfactory area of the septal region to the dorso-medial cortex of the hippocampus, entering this latter mainly, at least, on the medial side. In view of the fact that the medial surface of the hippocampus is mainly concerned, as far as one can judge from the impregnations of its cells (see discussion of the hippocampal cortex), with the reception of impulses, it seems quite probable that these fibers are concerned chiefly in carrying impulses from the parolfactory area to the cortex, i.e., they are parolfacto-cortical fibers. Since the medial parolfactry nucleus receives fibers from the medial olfactory tract and short fibers from the tuberculum olfactorium and since it is connected by way of the diagonal band of Broca with the lateral olfactory area and with the hypothalamus by way of the medial forebrain bundle, this nucleus probably serves as an olfacto-visceral correlation center and discharges the resultant of this correlation into the hippocampus by way of the parolfacto-cortical tract just described.

Tractus cortico-parolfactorius (fig. 17). Accompanying the lateral border of the fornix longus (see the account of the fornix beyond) as it swings ventralward from the hippocampus, there are relatively numerous fibers which enter the more dorsal portion of the lateral parolfactory nucleus and, spreading out, distribute to approximately all parts of this cell mass. Fibers can be traced from this nucleus passing out medialward and ventralward to join the medial forebrain bundle. Since the lateral side of the dorso-medial part of the hippocampus is concerned mainly with the discharge of nervous impulses (see the discussion of the dorso-medial part of the hippocampal cortex),

it is probable that the majority of fibers between the lateral parolfactory nucleus and the hippocampus conduct in the descending direction and that the nucleus functions as a place of synapse between the hippocampal cortex and the lower brain centers.

So far as the present data go they appear to suggest a division of labor between the medial and the lateral parolfactory regions (medial and lateral septal nuclei of some authors) and to suggest a motive for their differentiation, viz., that the medial nucleus is a way-station for ascending impulses going toward the hippocampus and the lateral nucleus is a similar station for descending impulses coming from the hippocampus. The writer is aware that the data are insufficient for a definite conclusion and that experimental researches or even more favorable Golgi material may prove these suggestions erroneous.

Commissures of the forebrain

There are two large commissures in the forebrain, the hippocampal commissure and the anterior commissure.

Commissura hippocampi (figs. 18, 19). The fibers of this commissure arise as axones of the projection cells of the hippocampus, which run ventralward and across the mid-line just above the anterior commissure. After crossing, some of the fibers appear to end in the nucleus commissuralis, but most of them pass dorsalward and end in synaptic relation with the cells of the opposite hippocampus. Thus the hippocampi of the two sides are put into connection with each other and enabled to work in a correlated way.

The commissura hippocampi has been the cause of much dispute among the earlier neurologists. Osborn ('87) identified it as the corpus callosum and for a time this interpretation was generally accepted. Adolf Meyer ('85) showed it to be the commissure of the medial and dorso-medial wall, which regions he identified as hippocampus. Elliot Smith ('03) claimed that in reptiles and monotremes there were no callosal fibers in the dorsal commissure. Johnston ('13a, pp. 402-404) is quite

certain from a series of experiments performed on the opossum that in this form there are callosal fibers in the hippocampal commissure. He believes that callosal fibers are present in that commissure in reptiles also, although he does not have the experimental proof for their presence there.

In the alligator, some of the fibers entering into the hippocampal commissure appear to come from the region of the general cortex and so to favor Johnston's conclusions, but of course nothing definite can be settled in this regard until some further degeneration experiments have been carried out. Unger, Kappers, DeLange, and others have described and figured the medullated fibers of the hippocampal commissure.

Commissura anterior (fig. 18). The following components of this commissure have been identified:

a. Stria terminalis fibers. The course of these fibers through the commissure is described under the account of the fiber systems (see account of stria terminalis pars commissuralis).

b. Fibers from the tractus olfactorius intermedius to the tuberculum olfactorium and the nucleus olfactorius anterior of the other side. These are myelinated.

c. Also, short fibers from the nucleus olfactorius anterior and the tuberculum olfactorium of one side to the corresponding centers of the other side.

d. So-called 'commissura epistriata' fibers (DeLange '11). These are included under the description of the stria terminalis fibers. This component consists of true commissural fibers of the stria terminalis, which connect the pyriform lobe and the nucleus of the lateral olfactory tract of the two sides of the brain, and of decussating fibers of other types.

Tract of the diagonal band of Broca

These fibers connect the region of the nucleus of the lateral olfactory tract with the parolfactory region and the nucleus commissurae hippocampi of the same side. These fibers pass ventrally of the basal forebrain bundles close to the surface of the brain. Caudalward many of the fibers end in the ventro-

medial nucleus and a few of them enter the anterior end of the nucleus preopticus. This fiber tract has been seen and more fully described by Johnston ('15, p. 407) in the brain of the turtle. Because of the deposit of silver on the outer surface of the Cajal material in this region, it has been impossible to study this fiber tract in the alligator as carefully as would be desirable. Apparently, however, it is made up, in part, of short fibers which form synapses among the cells of the diagonal band (see the description of this nucleus). The significance of this tract lies in the opportunity it gives for a close connection between the lateral and medial olfactory areas of the hemisphere (figs. 16 to 19).

Stria terminalis

This stria consists of two divisions.

The commissural portion (St. term. p. com., fig. 18). Slightly anterior to the level of the anterior commissure fibers may be seen passing from the region of the pyriform lobe, the nucleus of the lateral olfactory tract (particularly its dorsal portion), and the extreme ventro-lateral portion of the dorso-lateral area, directly medialward over the dorsal surface of the medial forebrain bundle (M. F. B.). Most of these fibers cross to the opposite side through the anterior commissure and distribute to the corresponding regions of the other half of the brain. Some of the fibers end in the bed nucleus of the anterior commissure of the same and the opposite side.

The preoptic portion (St. term. p. preop., figs. 19 to 21). This part is formed by fibers which distribute to the region of the pyriform lobe, the ventro-lateral part of the dorso-lateral area and the nucleus of the lateral olfactory tract from the posterior end of the region reached by the commissural portion of the stria terminalis to the caudal end of the basal portion of the hemisphere. This preoptic division turns medialward and caudalward, lying ventral to the posterior division of the lateral cortico-habenular tract and dorso-lateral and dorsal and in close relation with the olfactory projection tract of Cajal and its accompanying cell band—the interstitial nucleus. This preop-

tic portion of stria terminalis does not cross in the anterior commissure but passes caudad of it and distributes to the preoptic region of the same side.

Alveus

A large number of the alveus fibers arise as axones of the double pyramid and small projection cells of the hippocampus and run dorsalward then lateralward and then ventro-lateralward around the outer border of the ventricle to the pyriform lobe (figs. 15 to 21). They distribute during their course to the general cortex, the cortex of the pyriform lobe and at least to the anterior end of the nucleus of the lateral olfactory tract (the part which is a derivative of Johnston's small celled portion of the pyriform lobe). From the pyriform lobe and quite possible from these other regions, axones enter the alveus. Probably they distribute to the general pallium and hippocampal cortex.

A small number of alveus fibers at the anterior end of the forebrain swing outward between the hippocampus and the primordial neopallium and distribute along the outer surface of the latter. These association fibers between the two cortical areas have been very significant in determining the evolution of the primitive neopallium.

Fimbria

This is a term applied to the fibers which border the hippocampal cortex along its ventro-medial boundary (figs. 18 to 21). Behind the foramen of Monro these fibers also border the place of attachment of the choroid plexus. In the alligator fibers to the fornix, to the tractus cortico-habenularis medialis, and association fibers between the cortex of the pyriform lobe and the hippocampus are found in the fimbria.

Fibrae tangentiales

These are short association fibers which tie up the medial and the dorso-medial portions of the hippocampus (figs. 15 to 21). They

are on the superficial or pial side of the layer of cortical cells. Near the anterior end of the hemisphere short association fibers pass between the dorso-medial portion of the hippocampus and the general cortex.

These short superficial association fibers convey the nervous impulses which probably have operated in the course of the phylogeny to draw the cells of the primordial neopallium from the ventricular to the superficial position (neurobiotaxis; cf. the preceding discussion of the general cortex).

Fornix

In the fornix system of the alligator three parts have been distinguished. The first of these is the commissura hippocampi (commissura fornicis), which has already been described. The other parts are the columna fornicis and the fornix longus.

Columna fornicis (figs. 18 to 21, *F*). The fibers making up this division of the fornix are mainly axones of the double pyramid cells and small projection cells of the hippocampus. As has been said before, the fornix fibers join the hippocampal commissure and the tractus cortico-habenularis medialis. They swing first slightly lateralward and then medio-ventralward. Below the foramen of Monro the columna fornicis fibers separate trom those of the hippocampal commissure and, running caudad, enter the hypothalamus. They are accompanied by the fibers of the olfactory projection tract of Cajal (figs. 19 to 21).

The description here given agrees with the relations brought out by C. J. Herrick ('10). The myelinated fibers of the fornix have been described again and again by workers on the reptilian brain, among the number being Rabl-Rückhard ('81), Edinger ('88 and '96), C. L. Herrick ('93), Adolf Meyer ('92), Unger ('06), and DeLange ('11).

Fornix longus (figs. 16, 17, *F.L.*). This term is applied in mammals to a diffuse collection of fibers of mixed character passing in the medial wall of the hemisphere between the precommissural hippocampus (and adjacent parts of the cortex) and

the basal centers of the septum and hypothalamus. In the alligator there is a broad connection between the medial forebrain bundle near its anterior end and the overlying hippocampus which is probably in a general way comparable with the mammalian fornix longus. Since these fibers connect chiefly with the lateral or ventricular side of the layer of cortical cells, they probably are mainly descending projection fibers for the septum and hypothalamus.

Stria medullaris

The stria medullaris is made up of a number of fiber tracts running from secondary and tertiary olfactory centers to the habenula (figs. 11, 20, 21). The terminology here used follows Herrick ('10). The following components were identified and traced out:

1. *Tractus cortico-habenularis medialis* (figs. 18 to 20). This tract arises for the most part from the axones of the double pyramids and small projection cells of the hippocampus. Its fibers leave the hippocampus at the same level as those for the columna fornicis and the commissura hippocampi. All three bundles run ventralward together, the more lateral belonging to the tractus cortico-habenularis medialis, the intermediate ones to the columna fornicis, and the medial ones to the commissura hippocampi. After a time the commissural fibers run more toward the mid-line and become separated from the general fiber mass, while the cortico-habenular fibers turn dorso-lateralward, and, passing caudad into the diencephalon, enter the stria medullaris.

Part of the fibers of this medial cortico-habenular tract arise among the cells of the nucleus commissurae hippocampi. Scattered cells of this nucleus accompany the tract through the greater part of its course. Accordingly the tractus cortico-habenularis medialis receives impulses from the hippocampus of the same and the opposite side, impulses coming from the latter by way of the commissura hippocampi and its nucleus.

2. *Tractus cortico-habenularis lateralis anterior* (figs. 16 to 21). The fibers of the anterior division of the lateral cortico-haben-

ular tract arise from the more anterior part of the nucleus of
the lateral olfactory tract, from the cortex of the pyriform lobe
in that region, and possibly from the nucleus of the diagonal
band of Broca. The fibers run medialward in the ventral part
of the forebrain, mingling in part with the fibers of the diagonal
band of Broca, which lie on the ventral surface of the brain just
external to them. Near the medial border of the hemisphere
the anterior cortico-habenular tract turns dorsalward over the
ventro-medial nucleus and occupies a position in the angle be-
tween that nucleus and the lateral forebrain bundle (figs. 20,
21). In this angle it is joined by a fiber band which extends
along the medial surface to the caudal portion of the nucleus
ventromedialis among the cells of which nucleus a part of the
fibers can be traced (fig. 20). These two components of the
tract are joined on their medial surface, at this angle between
the ventro-medial nucleus and the lateral forebrain bundle, by
the lateral olfacto-habenular tract and the tracts run dorsalward
together and enter the stria medullaris and so reach the
habenula.

Accompanying the fibers of the anterior division of the lateral
cortico-habenular tract from the nucleus of the lateral olfac-
tory tract and the pyriform lobe is a small bundle of fibers aris-
ing from the same regions, passing dorsal to the ventro-medial
nucleus. Instead of entering the angle, however, between that
nucleus and the lateral forebrain bundle, this band of fibers
runs farther medialward and joins the medial forebrain bundle
(figs. 20, 21). It runs caudalward in this bundle. Its posterior
distribution is not certainly known as its fibers cannot be dis-
tinguished from others of the medial forebrain tract. Unless
it changes its relative position, however, it probably ends in
the hypothalamus, but nothing definite is known of its ending.
In its connections within the hemisphere and in its relative po-
sition in respect to the forebrain bundles, this fiber tract from
the pyriform lobe region shows several points in common with
the tractus pallii of lower forms (Herrick, '10). It is possible
that it and the olfactory projection tract of Cajal may be the

representatives of that tract in reptiles. It has been termed in this account, the ventral olfactory projection tract.

The portion of this tract which arises from the pyriform lobe and associated regions is evidently the same tract as that described by Kappers and Theunissen ('08, p. 225) for the lizard, Iguana, under the name tractus olfacto-habenularis (see figures 21 and 22 of their paper). Farther forward these authors describe it as turning lateralward to connect with the 'lateralen Lobusrinde' (fig. 20), which is apparently the pyriform lobe region of the present account.

There are probably other components of this fiber complex which have not been impregnated in the preparations studied.

3. *Tractus cortico-habenularis lateralis posterior* (figs. 20, 21). This large system of fibers arises from the nucleus of the lateral olfactory tract and the ventro-lateral part of the dorso-lateral area. Some of its fibers may arise from the overlying cortex of the pyriform lobe. These fibers pass medialward, at the same time sweeping dorsalward to avoid the area of distribution of the stria terminalis. At the lateral border of the thalamus they run parallel with and dorsally of the stria terminalis fibers (figs. 20, 21) and here they turn abruptly dorsalward to enter the stria medullaris thalami.

4. *Tractus olfacto-habenularis medialis* (figs. 20, 21). This tract arises from the more posterior portion of the nucleus preopticus, runs dorsalward medial to the medial forebrain bundle and turns forward and forms the most anterior part of the stria medullaris.

5. *Tractus olfacto-habenularis lateralis* (figs. 20, 21). This tract has its origin from the more anterior portion of the nucleus preopticus. It runs first lateralward on the extreme ventral surface of the brain ventrally of the basal forebrain bundles, then backward and dorsalward, joining the tractus cortico-habenularis lateralis anterior in the angle between the ventro-medial nucleus and the lateral forebrain bundle and passes dorsalward with it to enter the stria medullaris. (See description of tractus cortico-habenularis lateralis anterior for a further account of the relations.)

6. *Tractus olfacto-habenularis posterior*. This tract arises near the posterior end of the hemisphere from the nucleus of the lateral olfactory tract and the ventro-medial nucleus in the region illustrated in figure 12. It passes directly dorsalward into the stria medullaris.

Olfactory projection tracts

The entire secondary olfactory area is broadly connected with the hypothalamus by way of the medial forebrain bundle. Descending impulses are carried from the medial (septal) wall of the hemisphere through the tractus parolfacto-hypothalamicus (tr. septo-hypothalamicus of some other authors), as described beyond in the account of the medial forebrain bundle. The connections between the olfactory centers in the lateral wall of the hemisphere and the hypothalamus may in the aggregate be termed the olfactory projection tracts, following the usage of Ramón y Cajal. The application of the term projection tracts to these fibers finds its justification in the intimate relation between the secondary or basal olfactory centers and the olfactory cortex of the pyriform lobe in the lateral wall.

There are two of these tracts which enter respectively the ventral and the dorsal sides of the medial forebrain bundle (figs. 19 and 20), which together probably correspond approximately with the so-called tractus pallii of fishes and amphibians.

Ventral olfactory projection tract (figs. 16 to 19). This tract has already been mentioned in our account of the anterior division of the lateral cortico-habenular tract. It arises from cells of the pyriform lobe and the nucleus of the lateral olfactory tract. It runs with the anterior division of the lateral cortico-habenular tract until it reaches the ventral part of the medial forebrain bundle, which latter it accompanies caudad. It probably ends in the hypothalamus.

Olfactory projection tract of Cajal (figs. 19 to 21). The fibers of this olfactory projection tract pass directly dorsalward from the ventro-medial nucleus, then curve medialward and pass caudad lying dorsally of the forebrain bundles and between them

and the preoptic portion of the stria terminalis. Some of the fibers arise probably from cells of the interstitial nucleus and fibers from cells of the ventro-medial nucleus probably send collaterals into the interstitial nucleus. The fibers of this great olfactory projection tract as they swing medialward come into relation with the descending fibers of the columna fornicis and there turn sharply caudad and run with the latter bundle backward, medialward and ventralward to the mammillary body (figs. 20, 21). (Dr. C. J. Herrick first called the writer's attention to the fact that fibers of this olfactory projection tract join the fornix fibers and accompany them ventralward).

Ramón y Cajal ('11, vol. 2, pp. 722–723, fig. 462) has described and figured this tract and its associated nucleus in the mouse. Johnston ('15) described the tract in the turtle. He considers it to be the characteristic connection of his medial large celled nucleus of the amygdaloid complex (the ventro-medial nucleus of this description), but does not mention the interstitial nucleus which accompanies it.

Basal forebrain bundles

Medial forebrain bundle (figs. 9, 16, to 21, *M.F.B.*). This is the tractus septo-mesencephalicus of Unger and DeLange. It arises from the parolfactory (septal) nuclei and runs, accompanied by fibers of the fornix longus, medialward and ventralward until it meets the lateral forebrain bundle, which lies farther laterally. The two bundles can be distinguished from each other for a long distance because of a difference in the angles at which the fibers are running. Finally the two become closely mingled and it requires careful study to distinguish them, although such a differentiation is quite practicable. According to DeLange ('13) and Unger ('11) the medial forebrain bundle runs to the midbrain. In the alligator in material prepared by the Cajal method a part of the fibers appear to end in the hypothalamus (tractus olfacto-hypothalamicus of the literature), while others pass caudad to the midbrain (tractus olfacto-peduncularis).

There is no direct evidence in the material studied regarding the direction of conduction, but the probability is that impulses

pass in both directions. It serves, then, partly as a discharge path from the parolfactory areas (tractus parolfacto-hypothalamicus and tr. olfacto-peduncularis) and perhaps also from the tuberculum olfactorium, and partly as a pathway by which visceral impulses from the hypothalamic region may reach the medial parolfactory area (tractus hypothalamo-parolfactorius) and, either with or without a synapse there, the hippocampus. Fibers connecting the parolfactory areas and the hippocampus run on the medial and lateral borders of the medial forebrain bundle.

Lateral forebrain bundle (figs. 9, 16, to 21, 37, 45, 46, *L. F. B.*). This bundle is made up in part of axones arising from the projection cells of the striatum. It runs ventro-medialward, joins the medial forebrain bundle on its lateral side, and then passes caudad into the diencephalon. This is the tractus strio-thalamicus of DeLange and Unger.

Besides these components of the lateral forebrain bundle which carry impulses from the striatal region, there are fibers from the lateral and medial nuclei of the thalamus which run ventralward and join the other fibers of this bundle and then go forward to the striatum. These facts are known because axones or the cell bodies of the lateral nucleus and nucleus rotundus have been seen to join this bundle (tractus thalamo-striaticus, or thalamic projection tracts).

There is a second thalamo-striatal path which runs from the anterior nucleus of the thalamus to the ventro-lateral small celled part of the hemisphere (that part which is Johnston's nucleus caudatus). This has been described by Johnston, DeLange, and others.

GENERAL DISCUSSION

The problems of forebrain morphology and especially those dealing with the evolution of the cortical areas have always had a peculiar fascination for the comparative neurologist. The broad lines and many of the details of forebrain development throughout the vertebrate series have been brought out by such observers as Edinger, Elliot Smith, Johnston, Herrick, and Kappers.

It is with considerable hesitation that the writer has undertaken the analysis of the anatomical data given on the preceding pages. Insufficient time and knowledge and the lack of experience have been very clearly realized and the following statements are offered merely as suggestions or as possible interpretations of some of the changes occurring and the factors operating during forebrain evolution.

Following the type of interpretation of Edinger, Herrick, Kappers, and Johnston, centers of the alligator hemisphere may be classified under two general heads which may be subdivided as follows:

1. Centers dominated by olfactory impulses
    A. Basal centers
        1. Medial olfactory area
            Nucleus olfactorius anterior (in part)
            Nuclei of the septum (in part), or parolfactory nuclei
        2. Lateral olfactory area
            Pyriform lobe complex (in part)
            Amygdaloid complex (in part)
        3. Intermediate olfactory area
            Tuberculum olfactorium
            Nucleus olfactorius anterior (in part)
            Nucleus of the diagonal band
        4. Correlation centers between telencephalic and diencephalic regions
            Tuberculum olfactorium (in part)
            Parolfactory nuclei (in part)
            Nucleus commissuralis hippocampi
            Bed nucleus of the anterior commissure
            Nucleus preopticus
            Interstitial nucleus of Cajal
            Amygdaloid complex (in part)
    B. Cortical centers (archipallium of Edinger)
        1. Hippocampal formation
            Small celled non-laminated part of hippocampus (the primordium hippocampi of Johnston, '13 and '15)
            Dorso-medial cortex (primordial gyrus dentatus, Elliot Smith, '96, Meyer, '92, Levi, '04)
            Dorsal cortex (hippocampal cortex, subiculum of Johnston '13)
        2. Lateral cortex (pyriform lobe)
        3. General cortex (to some slight degree)
11. Centers dominated by ascending somatic impulses from the thalamus
    C. Basal centers
        1. Dorso-lateral area
        2. Intermedio-lateral area
        3. Ventro-lateral areas (comparable to corpus striatum of Johnston '15)

D. Cortical centers
    1. General cortex (in part)
        Primordial general cortex (a special portion of this area in close
        relation with the dorso-lateral area)

The basal olfactory centers of the telencephalon will be seen
to be separated into two broad groups. In the first group are
those of the medial, intermediate and lateral areas which serve
primarily as secondary olfactory centers. These are old in type,
having their representatives in the hemisphere from cyclostomes
(Johnston, '12, Herrick and Obenchain '13) up through the ver-
tebrate series to man. They were originally simply a place of
synapse and consequent redistribution of incoming olfactory
impulses.

The second group of basal olfactory centers includes those
which have developed within the hemisphere later in the phylo-
genetic history as a place of correlation between olfactory and
non-olfactory impulses. It is significant that some of the centers
(as for example the tuberculum olfactorium), judging from
their fiber connections, are both secondary olfactory nuclei
and correlations centers for olfactory and non-olfactory impulses.
It is the forward growth, then, of non-olfactory fibers from the
diencephalon into the secondary and tertiary olfactory centers
of the hemisphere which has given the impulse toward differ-
entiation to the telencephalon. These nuclei of the hemisphere,
which serve as correlation centers for the olfactory and non-ol-
factory impulses, represent the beginning of that higher differ-
entiation. Yet these basal centers do not form true cortex.
In the Amphibia (Herrick, '10) in the ventro-medial part of the
hemisphere, centers showing such type of correlation are pres-
ent and the medial forebrain bundle, which opens the possibil-
ity of connection between the olfactory centers and the visceral
centers in the hypothalamus, is well developed. In the dorso-
medial part of the hemisphere of Amphibia the material, which
is the primordium of the hippocampus, is present; it is under the
influence of olfactory fibers and, to some extent, of fibers of the
ventro-medial area of mixed function as just indicated. But
here no clearly developed cortex is found and it is not until the

basal olfactory and non-olfactory correlation areas are well developed, as in reptiles, that true hippocampal cortex begins to appear.

Johnston has emphasized the fact that the hippocampus is an olfacto-visceral center, although in a later paper ('15, p. 412) he has said that there are olfacto-visceral correlations in the subiculum as well. It is well to notice that these types of nervous impulses are not first assembled in the hippocampus. On the other hand, this cortex simply brings together material already correlated, partly in the hypothalamus and more completely within the basal telencephalic centers. Three types of centers concerned with olfactory impulses are represented then within the hemisphere.

1. Those basal centers concerned with the distribution of olfactory impulses and their summation and correlation among themselves.

2. Those basal centers concerned with the correlation of olfactory and non-olfactory impulses.

3. Those centers which receive impulses from correlation centers of the second type or from similar non-olfactory correlation centers and integrate these impulses. This integration of material already correlated is characteristic of the reptilian cortex. Into the hippocampus come impulses from the parolfactory area and the tuberculum olfactorium on the one hand, and from the pyriform lobe cortex by way of the alveus on the other hand.

In Amphibia (Herrick, '10) the primordium hippocampi occupies the dorso-medial portion of the medial wall of the hemisphere. This region has all the characteristic fiber tracts of the hippocampus (cf. Herrick, '10, p. 480) but there is no differentiated cortex in this region except possibly to a small degree in Anura, where there is a row of cells close to the surface of the ventro-medial wall which send out wide spreading dendritic processes among the incoming fibers and which resemble in cell characteristics those cells found in the alligator at the anterior end of the hippocampal formation.

In lower reptiles the dorso-medial area begins to form true hippocampal cortex. In the turtle, although, as has already been

pointed out (see discussion of hippocampus), there is a clearly defined arrangement of a considerable part of the hippocampal formation into definite cortex-like layers, these layers have not moved out from the ventricle as in higher forms, but still form a ventricular mass.

The hippocampal cortex of the alligator represents another step in advance in differentiation, for here the cortex has moved away from the ventricle and accompanying this differentiation has been the specialization, at least to a considerable extent, of its medial aspect to serve as the afferent side of the cortex and its lateral aspect to serve as the efferent side. One of the causes at least, for the outward migration of cells of the dorso-medial area to form the hippocampal cortex is probably to be found in the operation of the law of neurobiotaxis (Kappers, '14). According to this law, cell bodies tend to migrate along their dendrites toward their source of stimulation. The medial olfactory tracts and other tracts bearing afferent impulses to the hippocampus are on the medial surface of the hemisphere and the cells of the developing cortical layers move out toward the surface of the hemisphere in order that they may come into closer relationship with the incoming impulses.

To recapitulate, the following steps appear to have lead from the primordial hippocampal type to the relatively simple type of cortex found in part of the hippocampal area in the alligator. In Amphibia (Herrick, '10) the afferent and efferent fibers spread out all through the dorso-medial area. Following a higher differentiation of the diencephalic and telencephalic subcortical correlation centers, there is a higher differentiation in the dorso-medial area so that the arrangement of the cells into cortex-like layers, such as we find in the turtle, occurs. This second step is followed in other reptiles by a further specialization of a part of the hippocampal cortex, so that it has an afferent medial side and an efferent lateral one.

The non-olfactory diencephalic fibers, which enter the telencephalon for the purpose of forming correlations with the incoming olfactory impulses, are partly visceral and partly somatic in type. Those ascending from the hypothalamus by way of

the medial forebrain bundle to reach the medial wall of the hemi-
sphere carry mainly visceral impulses and the dominant, al-
though not the only, type of correlation in this wall is prob-
ably olfacto-visceral.

Impulses of a like kind reach the pyriform lobe region from
the hypothalamus by way of the ventral olfactory projection
tract (figs. 16 to 19).   Thus, this series of steps which seems to
have lead to the development of the hippocampus, can no doubt
be duplicated in the development of the cortex of the pyriform
lobe through the interrelation existing between the hippocampal
cortex and the pyriform lobe cortex on the one hand and the in-
terrelation between that latter cortex and the amygdaloid com-
plex on the other, although the details are not very well known.
In Amphibia (Herrick, '10) the dorso-lateral area of the hemi-
sphere receives lateral olfactory tract fibers and presumably is
the primordial material for the formation of pyriform lobe cortex
and perhaps for part of the amygdaloid complex.   Moreover,
in Amphibia, the somatic area is ventro-lateral in position and
receives and sends out fibers through the lateral forebrain bundle.
In reptiles, this somatic area has increased in size because of the
greater number of somatic fibers that it receives; for accompanying
the telencephalic changes there has been an increased growth and
differentiation of the thalamic regions, particularly of the lateral
portions which receive the fibers of the incoming optic and leminis-
cus systems.   This differentiation of the lateral part of the
thalamus (the neothalamus of Edinger) is correlated with an in-
crease in the number of fibers sent forward into the hemisphere
from this region.   This increase in the incoming fibers has lead
to a change in the nuclear pattern among reptiles as compared
with the pattern found among Amphibia.   Some of the for-
ward extending somatic fibers have begun to pass dorsalward
of the old limits of the striatum and in the turtle (Johnston, 15)
and in the alligator, perhaps in other reptiles also, the dorsal part
of the lateral wall is chiefly a somatic correlation center.   The
lateral and ventro-lateral portion of this dorsal wall, however,
is occupied by the cortex of the pyriform lobe and the anterior
part of the nucleus of the lateral olfactory tract.   The main part

of the latter nucleus is found on the outer surface of the ventral part of the lateral wall external to the striatum complex.

What the factors were which produced these changes in form relation between these amphibian and reptilian brains it is quite impossible at present to say. The following account is offered as a possible suggestion of some of the ways in which these changes were brought about. Even in Amphibia one would expect the lateral part of the dorso-lateral area to be particularly closely tied up with the olfactory tract, for there the dorsal division of the lateral olfactory tract ends (Herrick, '10, p. 523, fig. 40, *tr. olf. d. lat.*). In more highly differentiated forms the same process probably occurred which is known to have happened in other parts of the brain, namely, that part of the cells will migrate outward, away from the general cell mass, in order to form a special receptive center for the incoming olfactory fibers (a nucleus of the lateral olfactory tract) while the remainder will come less directly under their influence. In Amphibia (Herrick, '10) some thalamic somatic fibers reach the dorso-lateral area, although they are few in number compared with the olfactory fibers reaching that region. As one passes from amphibians to reptiles, there is a great increase in differentiation of the somatic thalamic regions, as has been said before, and this differentiation is accompanied by an increase in the number of somatic fibers sent forward into the hemisphere by way of the lateral forebrain bundle. Some of the somatic fibers, passing dorsalward of the old limits of the striatum come into synaptic relation with the neurones corresponding with the old amphibian dorso-lateral area. Part of such fibers will form synapses with olfactory fibers and so a somatic-olfactory center, whose later representatives are the amygdaloid complex and the pyriform lobe cortex, will be formed. Others of these somatic fibers come into synaptic relations with the more medially placed neurones of this dorso-lateral area (i.e., those neurones less directly under the influence of the olfactory fibers). The entrance of this new mass of somatic fibers and the resultant somatic correlation will lead to an increase in both the cell number and cell dfferentiation and in this way a non-olfactory somatic correlation center can well

grow up within areas primarily olfactory in type. With the entrance of a larger and larger number of somatic fibers within the area and the corresponding increase in the number and size of the cells, two changes in form relations occur in the lateral hemisphere wall. One change is the pushing outward and downward of the cell masses associated with the lateral olfactory tract so that they come to occupy secondarily a position superficial to the striatal region (the area occupied in the figures by the pyriform lobe and the nucleus of the lateral olfactory tract). The other change is the bulge medialward into the ventricle of the dorso-lateral somatic area which is so characteristic of the forebrains of the turtle and alligator.

The cortex of the pyriform lobe has arisen as a differentiation from the general cell mass of the forebrain which serves as a nucleus for the lateral olfactory tract. In the lower reptiles this cortex has appeared, although it is less differentiated than the olfactory cortex of the medial wall. Johnston ('16) has given very briefly some of the main features of the embryonic development of the lateral olfactory area and the cortex of the pyriform lobe in turtles. He finds the olfactory areas differentiating in the ventro-lateral part of the hemisphere and believes that the pyriform lobe cortex arises from cells of this region which have proliferated and perhaps migrated dorsalward, so that they came to lie external to the dorsal ventricular ridge. He is not certain, however, that they have not developed in situ. So far as the writer is aware, the history of the embryonic development of the pyriform lobe cortex in the alligator is unknown, but in all probability it is very similar to that of the development in the turtle. Of course, the question at once arises as to the factors operating to produce the specialized pyriform cortex from the general nucleus of the lateral olfactory tract and the reason for its new migration dorsalward (if that occurs as Johnston believes). In attempting to find a solution for the question one must look for the entrance into this region of fibers carrying a different type of impulse, for differentiation within an area is not dependent upon an increase in the number of fibers bearing the same sort of impulse but upon the introduction into the region of fibers bearing

a new type. Such a new type is introduced into the primordial pyriform lobe by the alveus, which carries the association fibers from the other developing cortical areas, particularly from the hippocampus. These impulses brought by the alveus are the resultants of a relatively high type of progressively advancing integration and give the physiological conditions which the writer has conceived of as being important in the development.

Attention has already been called to the presence of a large basal somatic area in the dorso-lateral region of the forebrain in at least some of the reptiles. This region has been named the dorso-lateral area (figs. 2, 7 to 10, 12, 16 to 19, 44 to 46, *Dl.A.*, in this paper. · A similar if not entirely homologous area has been termed the dorsal ventricular ridge by Johnston ('15) who in a recent paper ('16) has given an account of its embryological development. He finds that the dorsal portion of the embryonic brain in the turtle first gives rise by a process of cell proliferation to the general pallial cortex which then occupies a more superficial position. By a secondary proliferation from cells of the dorsal area, if the writer has understood Johnston ('16) correctly, the dorsal ventricular ridge is formed. The lateral portions are in relationship with the general pallial cortex and have the appearance of being an infolding of that area in adult material. In the adult turtle, the dorsal ventricular ridge extends to the caudal end of the hemisphere, showing throughout its extent this relationship with the general pallium. In the alligator the ridge of cells (termed here primordial general cortex) is found only in the anterior end of the hemisphere where it has practically the same relationships as in the turtle. Farther caudad the dorso-lateral area, of which the general cortex is a differentiation, is cut off from the latter region by the outward and downward growth of the ventricle. The dorsal and dorso-medial portions of this dorso-lateral area, however, receive somatic fibers throughout practically their whole extent as far as the caudal end of the area. Association fibers from the hippocampus and the pyriform lobe distribute not only to the general cortex but also to the primordial general cortex in the anterior end of the dorso-lateral area. These superficial tangential association fibers have probably been

responsible for the migration of the cells of the general pallium
from their original ventricular position to form a more super-
ficial cortical lamina, for their neurobiotactic influence (Kap-
pers) would have this tendency.

SUMMARY

To recapitulate, it appears to us that the following factors are
involved in giving the morphological form and typical functional
activity of the alligator forebrain:

1. This forebrain is very largely under the dominance of the
olfactory system.

2. Its differentiation into basal and cortical centers is due,
directly or indirectly, to the entrance of non-olfactory dien-
cephalic impulses.

3. These diencephalic fibers are partly for synaptic relations
with the olfactory fibers; and consequently basal centers for the
correlation of olfactory and non-olfactory impulses are present.
A variety in the type of the incoming diencephalic impulses has
lead to the differentiation of a number of different basal nuclei,
for it has not been the number of synapses through which an im-
pulse has passed, nor the number of fibers coming into a nucleus,
but the variety in the types of stimulation received which has
lead to the differentiation of the telencephalic centers.

4. In the lateral wall of the hemisphere the primordial striatum
is present, which is practically free from olfactory influence and
is under the influence of somatic fibers from the thalamus.   The
somatic area is larger than in lower forms and correlated with
this increase in size is an increase in size and differentiation of
the lateral part of the thalamus.

5. There are three primordial cortical areas represented and
they all have certain characteristics in common.   All of these
are primarily for the integration of impulses already correlated
in the basal centers or transmitted to them by way of association
fibers from the other cortical centers.   A certain proportion of
comparatively pure olfactory impulses enters the hippocampus
and the pyriform lobe; but, as has already been discussed, these

are primitive and, in themselves, insufficient for the formation of cortex.

Again, all the correlated material brought to each of these cortical areas contains olfactory and non-olfactory elements, the latter including visceral and somatic types. The differences in significance of the areas are due to a preponderance of a given type of correlation in each case. In the hippocampus the olfacto-visceral elements are very large and dominate the situation; in the pyriform lobe there is a considerable amount of correlation of the olfacto-visceral type, but there is a sufficient proportion of somatic impulses to give this lateral cortex a different physiological importance from that of the hippocampus. The olfacto-visceral types of correlation are small in the general cortex and the somatic types predominate.

The great significance of this general cortex in the alligator is the appearance of a somatic center having a high cortical type of integration. Nevertheless, since the general cortex is under tolerably direct olfactory influence from the adjacent hippocampal and pyriform cortex (and possibly from other sources), it cannot be regarded as fully differentiated neopallium, though it is undoubtedly the immediate precursor of that type of cortex.

LITERATURE CITED

Cajal, S. Ramón y   1909–1911   Histologie du Système Nerveux.   Paris, 2 vols.

Edinger, Ludwig   1888   Untersuchungen über die vergleichende Anatomie des Gehirns. 1. Das Vorderhirn. Abh. Senckenb. Ges., Bd. 15.
    1896   III. Neue Studien über das Vorderhirn der Reptilien Abh. Senckenb. Ges., Bd. 19.
    1899   IV. Studien über das Zwischenhirn der Reptilien. Abh. Senckenb. Ges., Bd. 20.

Gage, Susanna Phelps   1895   Comparative morphology of the brain of the soft-shelled turtle (Amyda mutica) and the English sparrow (Passer domesticus). Proc. Am. Micr. Soc., vol. 17, pp. 185–238.

Head, Henry and Holmes, Gordon   1911   Sensory disturbances from cerebral lesions. Brain, vol. 34.

Herrick, C. Judson   1910   The morphology of the forebrain in Amphibia and Reptilia. Jour. Comp. Neur., vol. 20, no. 5, pp. 413–547.
    1913   Some reflections on the origin and significance of the cerebral cortex. Jour. of Animal Behavior, vol. 3, no. 3, pp. 222–236.

HERRICK, C. JUDSON AND OBENCHAIN, JEANNETTE B   1913   Notes on the anat-
        omy of a cyclostome brain: Ichthyomyzon concolor.   Jour. Comp.
        Neur., vol. 23, no. 6, pp. 635–675.
HERRICK, C. L.   1890   Notes upon the brain of the Alligator.   Jour. Cincin-
        nati Soc. Nat. Hist., vol. 12, pp. 129–162.
        1891   Contributions to the comparative morphology of the central
        nervous system.   11. Topography and histology of the brain of certain
        reptiles.   Jour. Comp. Neur., vol. 1, pp. 14–37.
        1893   Contributions to the comparative morphology of the central
        nervous system.   11. Topography and histology of the brain of certain
        reptiles (Continued).   Jour. Comp. Neur., vol. 3, pp. 77–106, 119–140.
JOHNSTON, J. B.   1898   The olfactory lobes, forebrain, and habenular tracts of
        Acipenser.   Zool. Bull., vol. 1.
        1912   The telencephalon in cyclostomes.   Jour. Comp. Neur., vol. 22.
        1913   Nervus terminalis in reptiles and mammals.   Jour. Comp. Neur.,
        vol. 23, pp. 97–120.
        1913 a   The morphology of the septum, hippocampus, and pallial com-
        missures in reptiles and mammals.   Jour. Comp. Neur., vol. 23, pp.
        371–478.
        1915   The cell masses in the forebrain of the turtle, Cistudo carolina.
        Jour. Comp. Neur., vol. 25, no. 5, p. 393–468
        1916   The development of the dorsal ventricular ridge in turtles.
        Jour. Comp. Neur., vol. 26, no. 5, pp. 481–505.
        1916 a   Evidence of a motor pallium in the forebrain of reptiles.   Jour.
        Comp. Neur., vol. 26, no. 5, pp. 475–479.
KAPPERS, C. U. ARIENS   1906   The structure of the teleostean and selachian
        brain.   Jour. Comp. Neur., vol. 16, pp. 1–109.
        1914   Phenomena of neurobiotaxis in the central nervous system.
        XVIIth International Congress of Medicine.   London.
KAPPERS, C. U. ARIENS AND THEUNISSEN, W. F.   1908   Die Phylogenese des
        Rhinencephalons, des Corpus Striatum und der Vorderhirn-commis-
        suren.   Folia Neurobiologica, Bd. 1, pp. 173–288.
DELANGE, S. J.   1911   Das Vorderhirn der Reptilien.   Folia Neurobiologica,
        Bd. 5, pp. 548–597.
        1913   Das Zwischenhirn und das Mittelhirn der Reptilien.   Folia Neu-
        robiologica, Bd. 7, pp. 67–138.
        1913 a   L'Évolution Phylogénétique der Corps Strié.   Le Névraxe,
        vol. 14, pp. 105–122.
LEVI, GUISEPPE   1904   Sull' origine filogenetica della formazione ammonica.
        Archivio di Anat. e di Embriol., vol. 3, pp. 234–247.
MEYER, ADOLF   1892   Über das Vorderhirn einiger Reptilien.   Zeitsch. wiss.
        Zool., Bd. 55, pp. 63–133.
        1895   Zur Homologie der Fornix Commissur und des Septum lucidum
        bei den Reptilien und Säugern.   Anat. Anz., Bd. 10, pp. 474–482.
OSBORN, H. F.   1887   The origin of the corpus callosum.   Part 11, Morph.
        Jahr., Bd. 12, pp. 530–543.

RABL-RÜCKHARD 1878 Das Centralnervensystem des Alligators. Zeitsch. wiss. Zool., Bd. 30, pp. 336–373.
1881 Über das Vorkommen eines Fornixrudimentes bei Reptilien. Zool. Anz., vol. 4.

REESE, ALBERT 1908 The development of the American alligator. Smithsonian Misc. Coll., vol. 51, no. 1791, published by the Smithsonian Institution.
1910 Development of the brain of the American alligator. Smithsonian Misc. Coll., vol. 54, no. 1922, published by the Smithsonian Institution.

SHELDON, R. E. 1912 The olfactory tracts and centers in teleosts. Jour. Comp. Neur., vol. 22, no. 3, pp. 177–339.

SMITH, G. ELLIOT 1896 The fascia dentata. Anat. Anz., Bd. 12, pp. 119–126.
1903 On the morphology of the cerebral commissures in the Vertebrata with special reference to an aberrant commissure found in the forebrain of certain reptiles. Trans. Linnean Soc. of London, 2d series, Zool., vol. 8, pp. 455–500.
1908 The cerebral cortex in Lepidosiren. Anat. Anz., Bd. 33, nos. 20 and 21, pp. 513–540.
1910 The Arris and Gale Lectures. On some problems relating to the evolution of the brain. The Lancet, Jan. 1, 15 and 22.

UNGER, LUDWIG 1906 Untersuchungen über die Morphologie und Faserung des Reptiliengehirns. I. Das Vorderhirn von Gecko. Anat. Hefte, Bd. 31, Heft 94.
1911 Idem. II. Das Vorderhirn des Alligators. Sitzb. k. Akad. Wien, Math-nat. Klasse, Bd. 120, Abh. III.

ABBREVIATIONS

A., alveus
ax., axone
C.A., commissura anterior
C.H., commissura hippocampi
C.Hab., commissura habenularum
Cor.C., correlation cell
D.B., diagonal band of Broca
Dl.A., dorso-lateral area
D.pyr.C., double pyramid cell of hippocampus
F., fornix
F.B., forebrain bundles
Fib.tang., fibrae tangentales
Fim., fimbria
F.L., fornix longus
G.C., general cortex

glom., glomerulus
Glom.L., glomerular layer
Gran.L., granular layer
H., hippocampus
Hab., habenula
Hem., hemisphere
H.p.d., hippocampus, pars dorsalis
H.p.dm., hippocampus, pars dorso-medialis
Hypth., hypothalamus
I.Gran.L., inner granule layer
Interm.l.A., intermedio-lateral area
Inters.n., interstitial nucleus
Intr.C., intrinsic cell
L.F.B., lateral forebrain bundle
L.Gob.C., large goblet cell

L.P., lobus piriformis
M.C., mitral cell
M.C.L., mitral cell layer
M.F.B., medial forebrain bundle
N.acc., nucleus accumbens
N.ant.thal., nucleus anterior thalami
N.c.a., nucleus commissurae anterioris
N.c.h., nucleus commissurae hippo-campi
N.d.b., nucleus of the diagonal band of Broca
N.lat.thal., nucleus lateralis thalami
N.olf.ant., nucleus olfactorius anterior
N.parolf.lat., nucleus parolfactorius lateralis
N.parolf.med., nucleus parolfactorius medialis
N.preop., nucleus preopticus
N.tr.olf.lat., nucleus tractus olfactorius lateralis
N.vent.med., ventro-medial nucleus
O.Gran.C., outer granule cell
O.Gran.L., outer granule layer
Olf.B., olfactory bulb
Olf.C., olfactory crus
Olf.proj.tr.(Cajal), olfactory projection tract of Cajal
Op.ch., optic chiasma
Op.tr., optic tract
P., pulvinar
Plex.L., plexiform layer
Prim.G.C., primordial general cortex
Prim.h., primordial hippocampus
Proj.C., projection cell of the ventro-lateral area
S.Gob.C., small goblet cell
S.proj.C., small projection cell
St.C., stellate cell
St.med., stria medullaris

St.term.p.com., stria terminalis pars commissuralis
St.term.p.preop., stria terminalis pars preopticus
Taen.c., taenia chorioidea
Taen.f., taenia fornicis
T.olf., tuberculum olfactorium
Tr.cort.hab.lat.ant., tractus cortico-habenularis lateralis anterior
Tr.cort.hab.lat.post., tractus cortico-habenularis lateralis posterior
Tr.cort.hab.med., tractus cortico-habenularis medialis
Tr.cort.parolf., tractus cortico-parolfactorius
Tr.olf., tractus olfactorius
Tr.olf.cort., tractus olfacto-corticalis
Tr.olf.hab.lat., tractus olfacto-habenularis lateralis
Tr.olf.hab.med., tractus olfacto-habenularis medialis
Tr.olf.hab.post., tractus olfacto-habenularis posterior
Tr.olf.interm., tractus olfactorius intermedius
Tr.olf.lat., tractus olfactorius lateralis
Tr.olf.med., tractus olfactorius medialis
Tr.parolf.cort., tractus parolfacto-corticalis
Tr.tub.cort., tractus tuberculo-corticalis
Vent.olf.proj.tr., ventral olfactory projection tract
Vl.A.(l.c.), ventro-lateral large celled area
Vl.A.(s.c.), ventro-lateral small celled area

Fig. 1 The brain of Alligator mississippiensis, as seen from the dorsal surface. Drawn from a specimen 55 cm. long. × 3.

Fig. 2 A lateral view of the same specimen as in figure 1. A part of the lateral wall has been removed so as to expose the lateral ventricular surface of the dorso-lateral area. × 3. The line A–A represents the plane of section of figure 44; the line B–B that of figure 45.
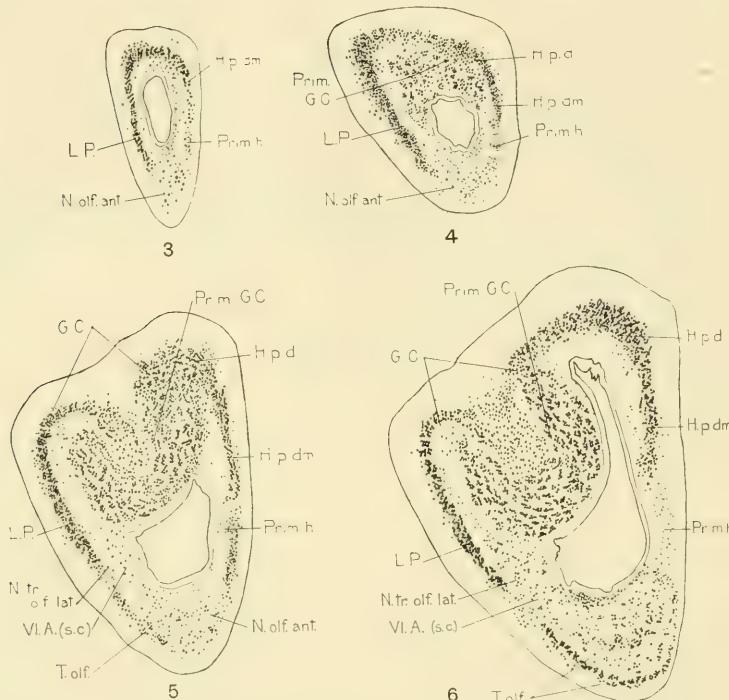
Figs. 3–12 A series of transverse sections through the hemisphere of Alligator mississippiensis. Toluidin blue. × 19. The serial numbers of the sections figured are appended to the descriptions.

Fig. 3 Section through the posterior part of the olfactory crus showing the anterior part of the pyriform lobe and the hippocampus (14 : 286)

Fig. 4 Section slightly caudad to the preceding, showing the primordium of the general cortex (16 : 318).

Fig. 5 Section illustrating the characteristic appearance of the general cortex (18 : 353).

Fig. 6 Section somewhat caudad to the preceding (19 : 370).

Fig. 7 Section through the posterior part of the primordium of the general cortex, showing the basal nuclei of the lateral and medial walls in that region (22 : 416).

Fig. 8 Section slightly caudad to figure 7 (23 : 436).

7

8
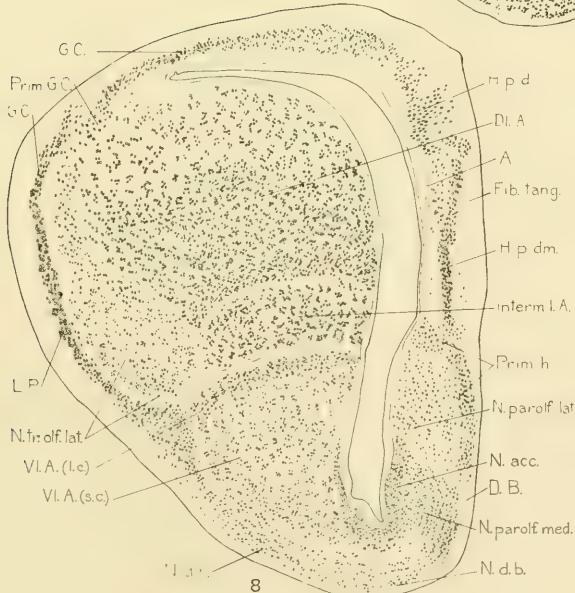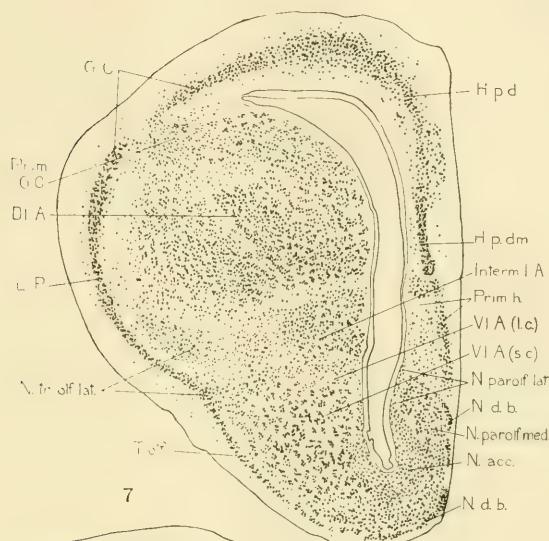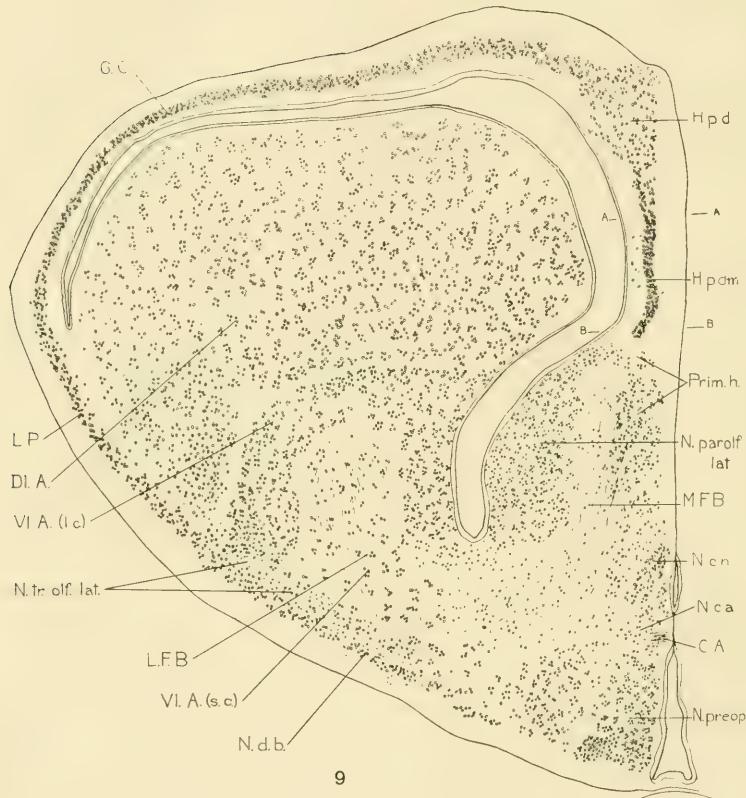
Fig. 9   Section through the level of the diagonal band of Broca, showing the relations of the parolfactory nucleus and primordium hippocampi (27 : 1, 4). (*A–A′* and *B–B′* show the orientation of figure 30.)

Fig. 10   Section through the anterior end of the thalamus.   Note the relative positions of the ventro-lateral, small-celled area and the nucleus anterior thalami (29 : 3, 1).

Fig. 11   Section through the nucleus lateralis thalami.   Note the large size of the cells   (31 : 2, 3).

10

11

12
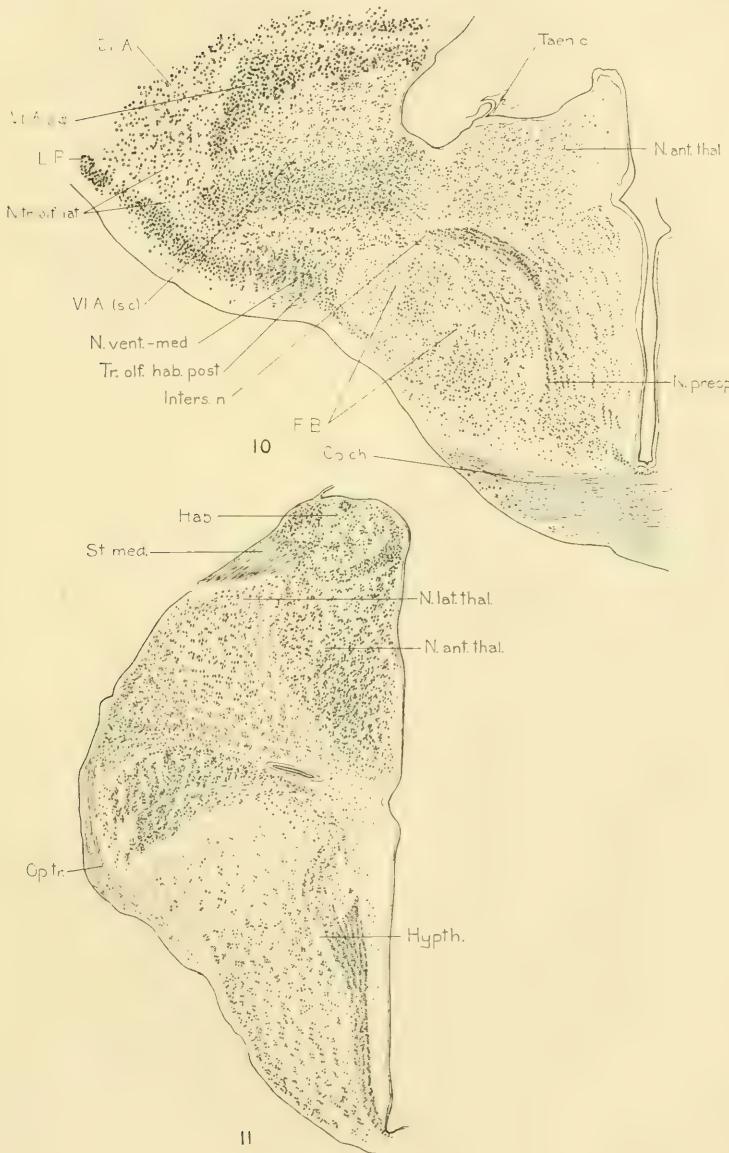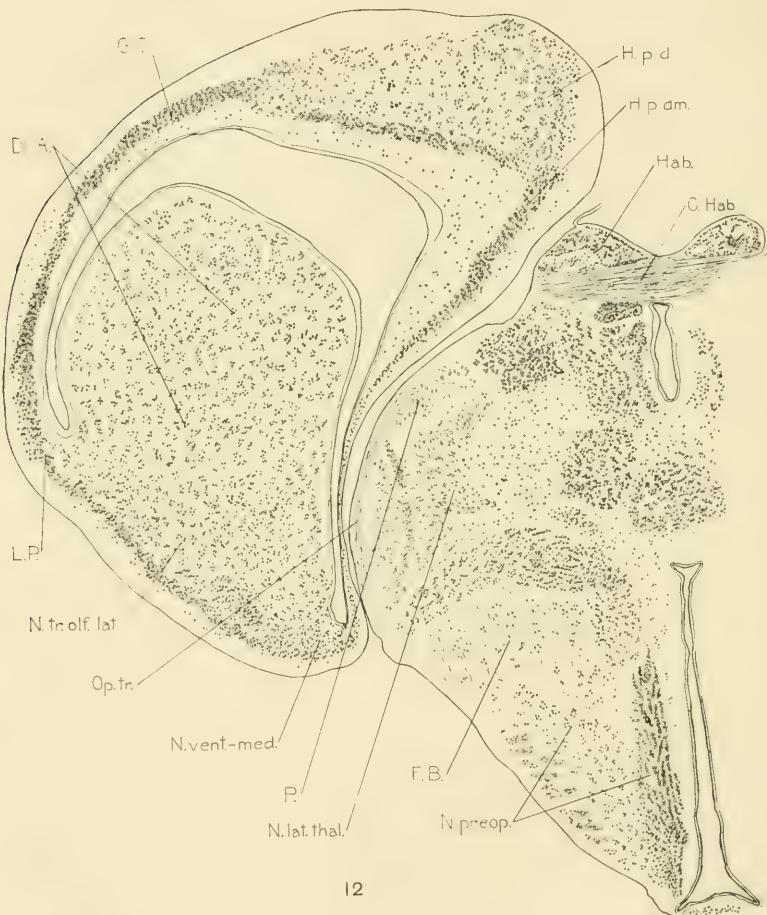
Fig. 12 Section through the habenular commissure. (32 : 3, 3).

Figs. 13–21 Transverse sections prepared by the Cajal method. Sections from two different series were used in preparing this series of drawings. ×13.

Fig. 13 Cross section through the left olfactory bulb anterior to the olfactory ventricle. The characteristic groupings of the internal and external granule cells and the ring-like arrangement of the mitral cells are clearly shown. The incoming fila olfactoria and the glomeruli are shown in the figure (3 : 3, 2).

Fig. 13 labels: Tr. olf. lat., C. Gran., Gran., Tr. olf. med., M.C.L., G. com., 13

Fig. 14 labels: Tr. of med., L.P., H.p. dm, Tr. olf. lat., N. olf. ant., Tr. olf.-cort. + Tr. tub. cort., Tr. olf. interm., 14

Fig. 15 labels: H.p.d., L.F.B., Prim. G.C., A., Dl. A., H.p. dm, Tr. olf. lat., Tr. tub. cort., L.P., T. olf., Tr. olf.-lat. + Tr. olf. interm., 15

Fig. 16 labels: H.p.d., Dl. A., G.C., L.P., A., Fib. fang., H.p. dm, Tr. parolf. cort, M.F.B. + F.L, N. parolf. lat., N. parolf. med., N. acc., N. tr. olf. lat., Tr. cort. hab. lat. ant. + Vent. olf. proj. tr., Tr. olf. lat., L.F.B., D.B., N. d.b., 16

Fig. 17 labels: H.p.d., Dl. A., Fib. fang., G.C., A., L.P., H.p. dm, Tr. parolf. cort., Tr. cort. parolf., L.F.B., A., Tr. olf. lat., N. tr. olf. lat., Tr. cort. hab. lat. ant. + Vent olf. proj. tr., F.L. + M.F.B., N. parolf. lat., N. parolf. med., Vl. A. (l.c.), Vl. A. (s.c.), D.B., N. d.b., 17

Fig. 14 A transverse section through the posterior part of the olfactory crus where it is broadening out into the hemisphere (3 : 254).

Fig. 15 A transverse section through the right hemisphere at the anterior end of the neopallial primordium (8 : 3, 3).

Fig. 16 A section near the anterior end of the medial forebrain bundle, *M. F. B.* (12 : 4, 1).

Fig. 17 A section a short distance anterior to the hippocampal commissure (14 : 2, 3).
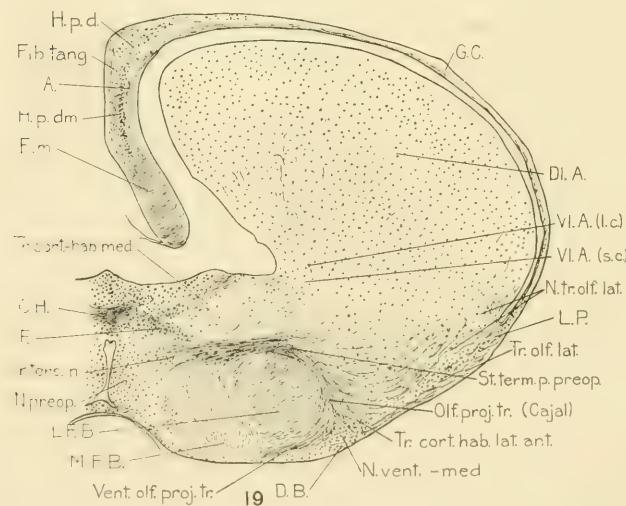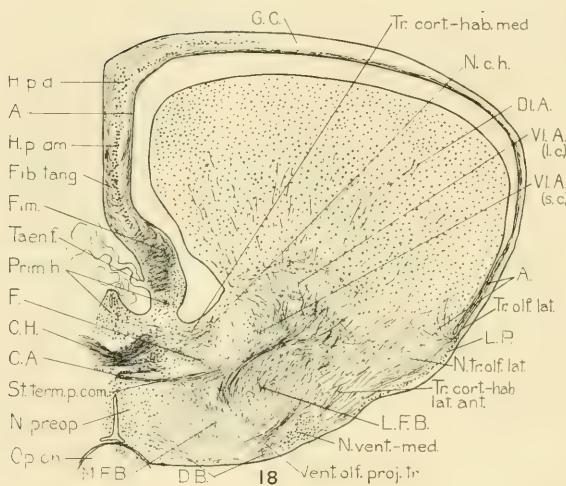
Figs. 18–21 These figures were drawn from a transverse series prepared after the Cajal method and loaned by Dr. P. S. McKibben. × 13.

Fig. 18 A section through the anterior part of the hippocampal commissure (11 : 780).

Fig. 19 A section through the posterior part of the hippocampal commissure and the beginning of the stria medullaris (11 : 788).
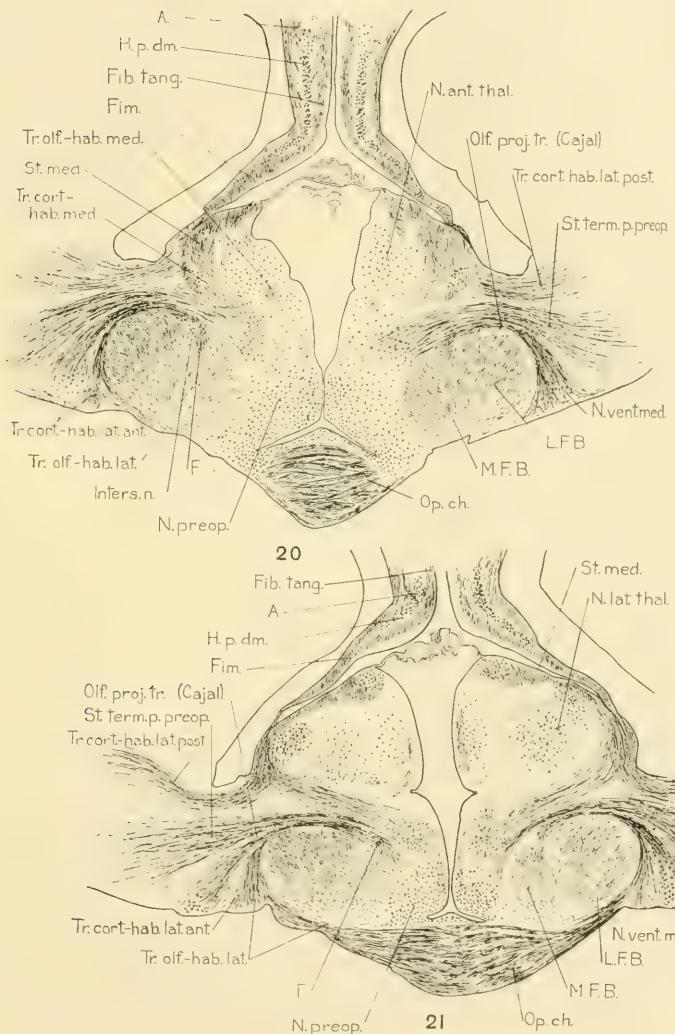
Fig. 20 Section through the anterior part of the thalamus, showing the relations of the fiber tracts (12 : 805).

Fig. 21 Section through the anterior part of the habenula (12 : 823).
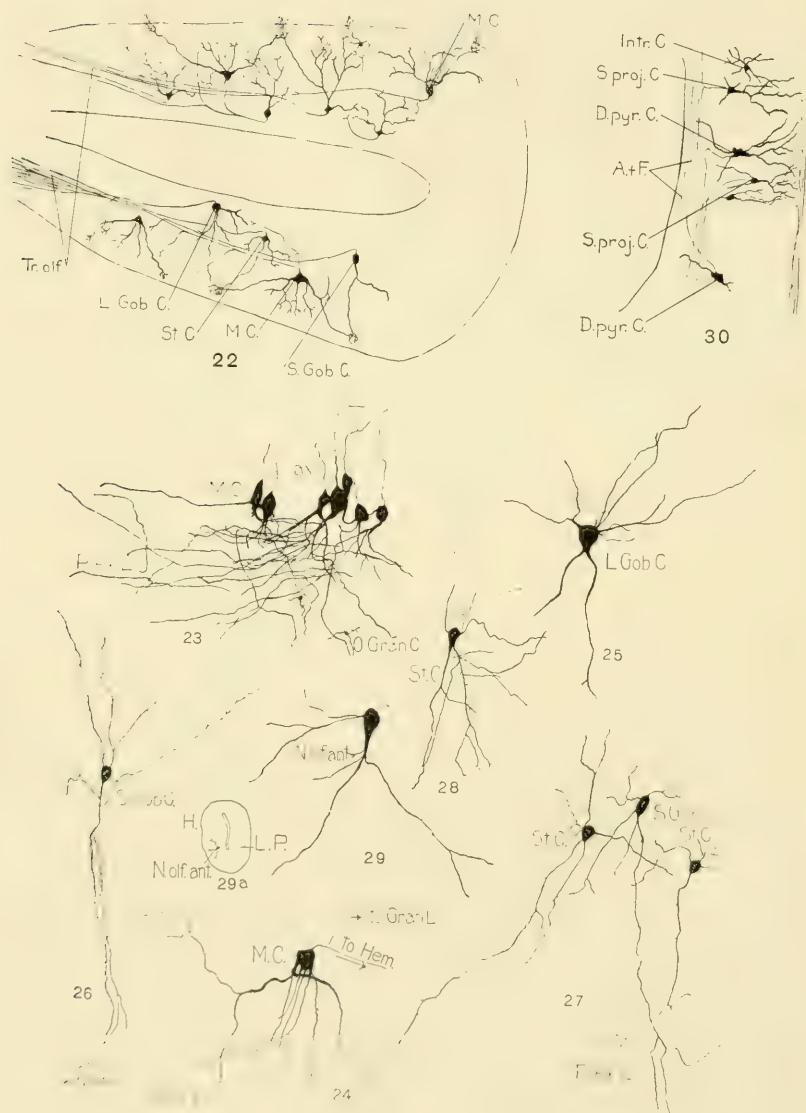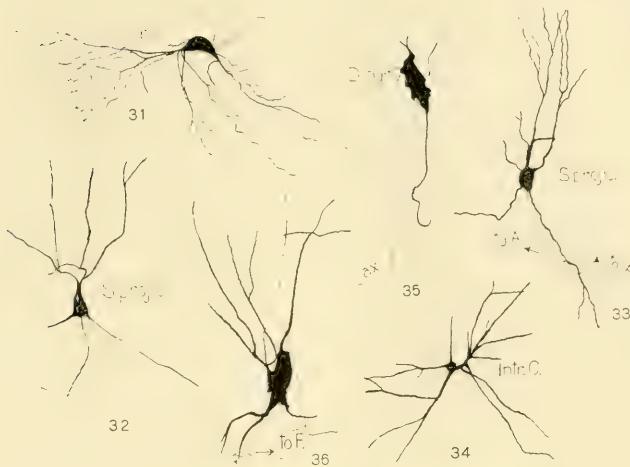
Figs. 22–43 Characteristic cells from various parts of the forebrain and thalamus of Alligator mississippiensis as seen in Golgi preparations. ×90.

Fig. 22 A diagramatic sketch of the positions and relations of the various cell types found in the olfactory bulb.

Fig. 23 Small mitral cells of the olfactory bulb (Gl : 60).

Fig. 24 Large mitral cell of the olfactory bulb (Gl : 73).

Fig. 25 Large goblet cell of the olfactory bulb (Gl : 64).

Fig. 26 Small goblet cell of the olfactory bulb (Gl : 55).

Fig. 27 Group of internal granule cells of the olfactory bulb. Note that one of the stellate cells sends its dendrites down into the plexiform layer and into the region, at least, of the glomeruli. The other does not send its dendrites outward beyond the mitral cells (Gl : 55).

Fig. 28 A small stellate cell of the olfactory bulb (Gl : 96).

Fig. 29 A goblet cell of nucleus olfactorius anterior (Gl : 103).

Fig. 29a A diagram showing the orientation of figure 29.

Fig. 30 A diagram showing the orientation of the hippocampal cells. The positions of the hippocampal cells figured (figs. 31–36) are shown here.

Fig. 31 Correlation cell found in the dorsal part of the hippocampus at the anterior end of the hemisphere (Gl : 104).

Figs. 32 and 33 Small projection cells of the dorso-medial part of the hippocampus (Gl : 139; Gl : 140).

Fig. 34 Intrinsic cell of the hippocampus (Gl : 140).

Figs. 35 and 36 Double pyramid cells. These are the specialized derivatives of projection cells of the dorso-medial portion of the hippocampus. The cells figured are probably imperfectly impregnated (Gl : 140; Gl : 139).
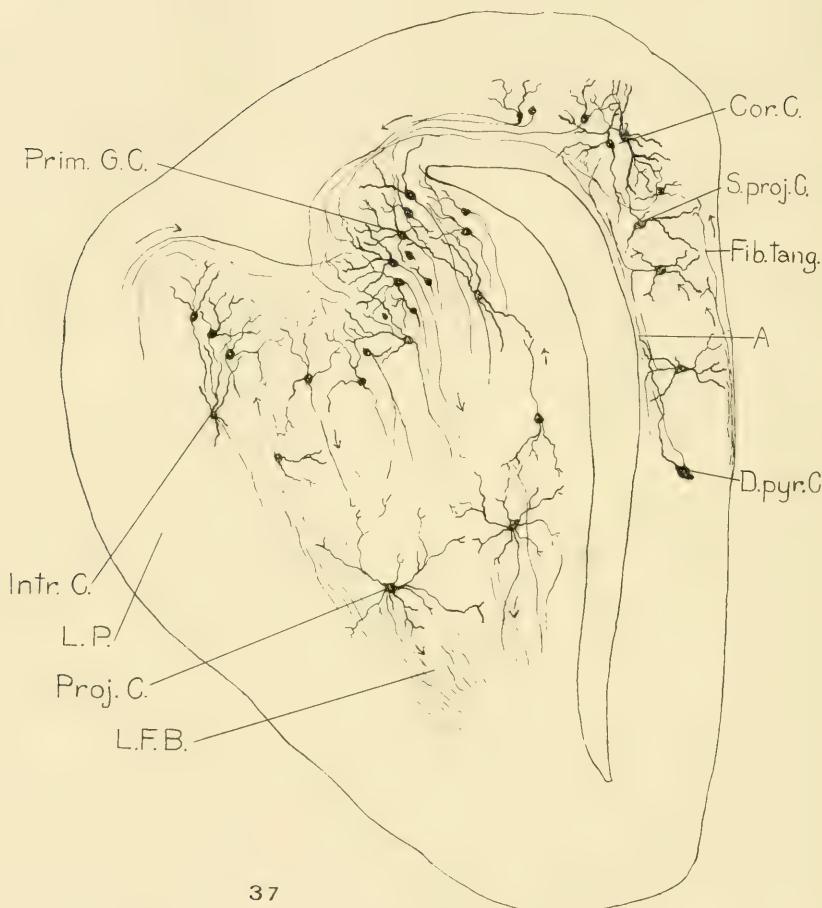
37

Fig. 37   This is a diagram of a transverse section through the hemisphere at the level of the primordial infolding.   The cells of this primordial general cortex are round or goblet shaped (fig. 40) and have their dendrites directed outward and their axones inward and downward into the striatum.   The axones come into relationship with the projection cells of the striatum, and, after a synapse, the impulse is carried by the axones of these projection cells through the lateral forebrain bundle to the lower centers.   Impulses reach the primordial general cortex from the hippocampus, the pyriform lobe and the thalamus (by way of the lateral forebrain bundle).   The interpolated neurone (*Intr.C.*) pictured in the diagram was not brought out very clearly in the Golgi sections, for, although neurones of that type were seen in the sections, they were never clear enough for high power drawings.   Several types of neurones can be distinguished in the toluidin blue sections and the cell labeled 'intrinsic cell' is a guess at one of their probable functions.
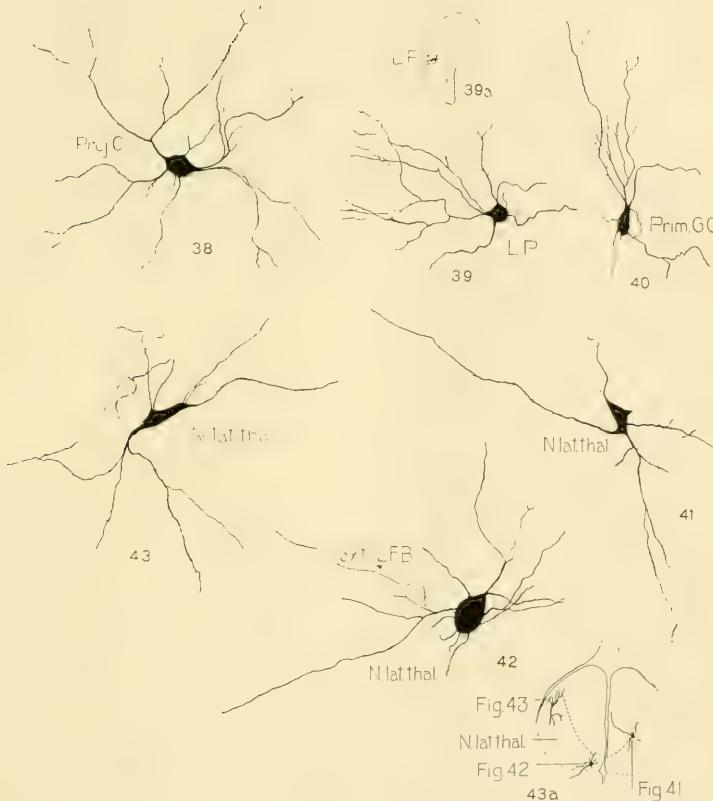
Fig. 38   Projection cell of the ventro-lateral area.   For orientation see figure 37 (Gl : 126).

Fig. 39   Cell from the anterior part of the pyriform lobe (Gl : 99).

Fig. 39a   Diagram for the orientation of figure 39.

Fig. 40   Cell from the primordial general cortex (Gl : 105).   For orientation see figure 37.

Figs. 41–43   Cells of the nucleus lateralis thalami   (Gl : L60;   Gl : 159; Gl : 159).

Fig. 43a   Diagram for the orientation of figures 41–43.
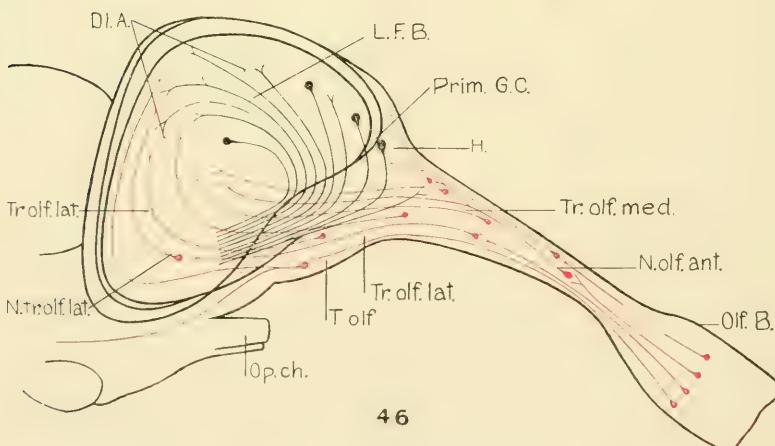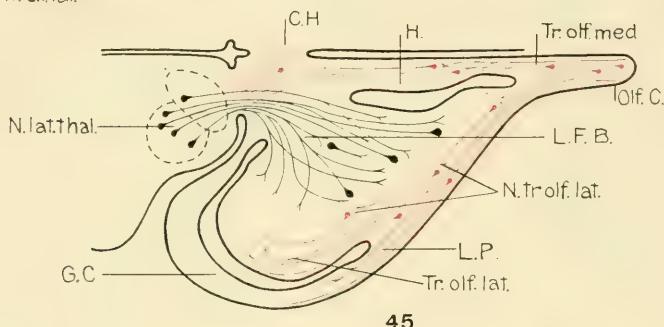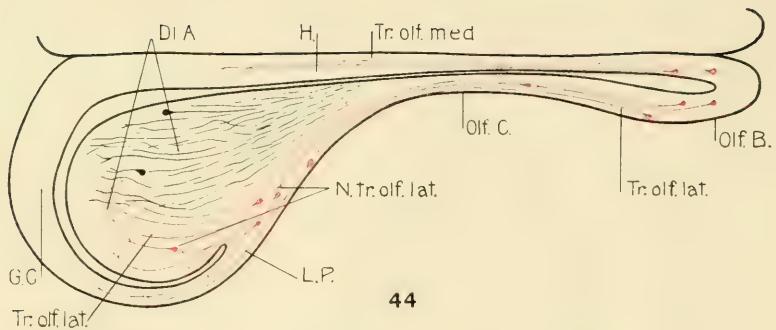
Fig. 44 A diagram of the connections of the olfactory tracts and the lateral forebrain bundle of the alligator, based on a longitudinal section of the hemisphere in the plane indicated by the line A-A' of figure 2. The olfactory tracts are printed in red, the lateral forebrain bundle in black.

Fig. 45 A diagram similar to figure 44, but taken farther ventral and in a somewhat different plane, passing through the level of the hippocampal commissure as indicated by the line B-B' of figure 2.

Fig. 46 A diagram of a longitudinal section through the forebrain of the alligator taken in a parasagittal plane, to illustrate the relations of the olfactory and somatic centers. Olfactory fibers red, somatic fibers black.

402

THE NUMBER, SIZE AND AXIS-SHEATH RELATION OF THE LARGE MYELINATED FIBERS IN THE PERONEAL NERVE OF THE INBRED ALBINO RAT— UNDER NORMAL CONDITIONS, IN DISEASE AND AFTER STIMULATION

M. J. GREENMAN

The Wistar Institute of Anatomy and Biology

In a study of regenerated peripheral myelinated nerve fibers and their controls (Greenman, '13) the question arose as to whether peripheral myelinated nerve fibers undergo changes in sectional area or changes in the axis-sheath relation during excessive physical exertion or in diseased animals.

Tashiro ('13) reported that resting nerve fibers, in vertebrates and invertebrates, warm blooded and cold blooded animals, give off CO_2; that nerve fibers increase their production of CO_2 about $2\frac{1}{2}$ fold when stimulated by an electrical, chemical, thermal or mechanical stimulus.

He concludes (1) that the nerve fiber has a metabolism and that this metabolism is modified by the state of excitation, (2) and that the increased CO_2 production accompanying stimulation can be used as a new criterion for protoplasmic excitability.

The experiments here described were conducted for the purpose of measuring, if possible, any morphological changes which might take place in a nerve fiber by reason of some general pathological conditions or by reason of activity and also of obtaining some information as to the origin of the myelin, its significance or the influences which increase or diminish it.

In these experiments the observations have been confined to the large myelinated nerve fibers of the right and left peroneal nerves of the inbred albino rat.

PRELIMINARY TESTS

A preliminary test was made upon two female gray rats (Mus norvegicus) of practically the same body weight, 236.5 and 226.5 grams, respectively. One rat, No. 323, was used as a control while the other, No. 322, was placed in a revolving cage operated by a motor and exercised continuously for four hours. At the end of this period the animal was given water but no food and left in the cage until the following day when the cage was again started and the animal exercised for a further period of fifteen minutes. At the end of this brief period the animal died.

The unexpected death of the animal complicated the test to some extent, since from previous experience with rats in revolving cages the amount of exercise given this animal should not have resulted in death. It is possible that the animal was unhealthy, though no external signs of disease were noted. No autopsy was made. However, as the object of the test was to detect changes in nerve fibers due to fatigue, I proceeded to remove the right peroneal nerve for comparison with that of the control animal.

Following the method of previous work on this nerve, 10 mm. of the right peroneal nerve just distal to the sciatic bifurcation were excised, in both the exercised (No. 322) and the control animal (No. 323), fixed in 1 per cent osmic acid, embedded in paraffine and cut into sections 7 micra thick. The sections from the middle zone of each nerve were examined. The number of myelinated fibers was determined and the sectional areas of the twenty largest fibers were measured.

In making these determinations Hardesty's ('99) method for counting fibers was employed; after making a photograph of each section to be counted, each fiber was automatically recorded and counted by pricking a hole in each fiber image of a photographic print, while the original section was observed under a Zeiss 2 mm. apochromatic objective with a No. 4 compensating ocular and a tube length of 160 mm.

The largest fibers of each section were selected by carefully going over each successive zone of the section. The sectional

areas of fibers, axis and sheath were determined by measuring with a compensating planimeter an outline of each fiber and of its contained axis, magnified 4000 diameters. These outlines were drawn on finely ground glass in the plate holder end of a specially constructed rigid camera. A Zeiss 2 mm. apochromatic objective with No. 4 compensating ocular constituted the optic apparatus of the drawing camera.

In order to keep the personal factor as low as possible all counts and all planimeter records were made by my assistant, while all drawings were made by myself.

For the skillful technical work and the accuracy of the counts and planimeter determinations I am indebted to Miss F. Louise Duhring.

Table 1 presents the summarized data of this preliminary test.

TABLE 1

NUMBER	WEIGHT AT TIME OF KILLING	NUMBER OF FIBERS	AVERAGE SECTIONAL AREA OF 20 LARGEST FIBERS		
			Fiber	Axis	Sheath
322 ♀ (exercised).............	226	1996	84.1	35.3	48.8
323 ♀ (control)...............	236	2054	105.6	40.3	65.2

The control fibers average 20.4 per cent larger than the fibers of the exercised animal.

The average axis-sheath relation in the control fibers is 38 per cent axis to 62 per cent sheath, while the average axis-sheath relation in the exercised animal is 42 per cent axis to 58 per cent sheath, showing a very slight difference in the percentage of sheath.

This preliminary test suggested that peripheral nerve fibers may be affected in area of section by excessive exercise and that possibly the axis-sheath relation may be thereby modified. However, as the animals were not known to be of the same litter, and as the limits of individual variation as to fiber areas were unknown, the test could only be regarded as an encouragement to pursue the work further.

A second test was therefore made using, in this case, an albino rat suffering from so-called 'pneumonia.' This animal had dropped from 264 to 184 grams in body weight in 66 days. Autopsy showed badly infected lungs with hemorrhagic areas and pus cavities.

A brother from the same litter was used as a control for this animal. The control animal had increased in body weight from 267 to 315 grams during the same period of 66 days. Autopsy negative.

TABLE 2

NO.	AGE	WEIGHT AT TIME OF KILLING	NUMBER OF FIBERS IN PERONEAL NERVES		AVERAGE SECTIONAL AREA OF FORTY LARGEST FIBERS IN SQUARE MICRA					
			Right	Left	Right			Left		
					Fiber	Axis	Sheath	Fiber	Axis	Sheath
352♂	·251 (pneumonia)	184	2240	2230	100.2	39.6	60.6	122.2	45.9	76.3
					Combined average of right and left					
					Fiber 111.2 Axis 42.7 Sheath 68.4					
353♂	251 (control)	315	2240	2296	124.9	46.8	78.1	108.8	43.1	65.7
					Combined average of right and left					
					Fiber 116.8 Axis 44.9 Sheath 71.9					

Table 2 gives the summarized records of the examinations made of both right and left nerves from both the 'pneumonia' animal and its control. In this case the technique followed was the same as in the first test. Forty of the largest fibers of each nerve were measured in this instance, giving the sectional area of the entire fiber, its axis and its sheath in square micra.

It will be seen from table 2 that in the diseased animal the average sectional area of the 40 largest fibers from the right nerve is less, while the average sectional area of the 40 largest fibers from the left nerve is greater than in the corresponding fibers of the control animal. If the averages from the right and

left nerve fibers of each animal are combined it will be seen that the fibers of the diseased animal are slightly less in area than those of the control animal. The difference, (5.6 square micra) however, is slight and probably insignificant. The axis-sheath relation in both animals is the same—38.5 per cent axis to 61.5 per cent sheath.

This test left the matter in doubt, but it seemed desirable to examine other cases of diseased animals.

'PNEUMONIA' RATS

For this further work, three groups of so-called 'pneumonia' rats were examined—each group being controlled by a healthy animal from the same litter.

TABLE 3

Series 14. Pneumonia rats and controls from same litters

Pneumonia rats

NUMBER	SEX	LITTER	AGE	STRAIN	PREVIOUS MAXIMUM WEIGHT	WEIGHT WHEN KILLED	AUTOPSY	NUMBER OF FIBERS		AVERAGE SIZE OF FORTY LARGEST FIBERS IN SQUARE MICRA					
										Right peroneal			Left peroneal		
								Right	Left	Fiber	Axis	Sheath	Fiber	Axis	Sheath
352	♂	7	251	Ext.-inbred*		184	Pneumonia	2240	2230	100.2	39.6	60.6	122.2	45.9	76.3
424	♀	29	335	Ext.-inbred	213	177	Pneumonia	2122	2000	137.3	57.2	80.1	161.2	66.7	94.4
425	♂	29	335	Ext.-inbred	395	282	Pneumonia	2145	2006	133.5	53.7	79.8	135.9	55.6	80.4
445	♂	35	454	Inbred	342	201	Pneumonia	2047	1988	128.2	53.0	75.1	119.3	47.9	71.4
447	♂	35	454	Inbred	331	292	Pneumonia	2040	1916	115.4	47.5	67.9	132.9	57.4	75.5
Averages.............................								2119	2028	122.9	50.2	72.7	134.3	54.7	79.6
											40%	60%		40%	60%

Control rats

353	♂	7	251	Ext.-inbred		315	Neg.	2240	2296	124.9	46.8	78.1	108.8	43.1	65.7
426	♂	29	335	Ext.-inbred	355	347.5	Neg.	2122	2165	139.8	52.5	87.3	130.8	52.2	78.5
446	♀	35	454	Inbred	248	208	Neg.	1973	1863	109.9	44.4	65.4	127.5	55.1	72.4
Averages ....,.............								2111	2108	124.9	47.9	76.9	122.3	50.1	72.2
											38%	62%		41%	59%

* Extracted inbred albino rat.

The results of these examinations are given in table 3. In this table the animals are arranged in the order of their increasing age. In all four pneumonia cases where the comparison can be made it will be observed that there was a marked loss from a previous maximum body weight, due most likely to the disease.

The normal weight for King's inbred strain of albino rats is 313.8 grams at 243 days, 332.3 grams at 334 days, and 358.7 grams at 455 days of age. These weights are from H. D. King's unpublished records.

It will be seen that the rats were all very much under normal weight at the time of killing.

If, for greater accuracy, we omit from our consideration the females No. 424, a 'pneumonia' animal, and No. 446, a control animal, and compare the average number of fibers from the right and left peroneal nerves of No. 352, a 'pneumonia' animal with the average number of fibers from the right and left peroneal nerves of No. 353, its control, we find the 'pneumonia' animal presents an average of 2235 fibers while its control gives an average of 2268 or 33 more fibers. If, in like manner, we compare the average number of fibers from No. 425 a 'pneumonia' animal with the average number from No. 426, its control, we find the average in the 'pneumonia' animal to be 2075 fibers while the average of its control is 2143 or 68 more fibers.

When we compare the average sectional areas of the fibers of the right peroneals of the 'pneumonia' rats with the corresponding averages of their controls, we note that these averages are practically the same, while in case of the left side the 'pneumonia' rats show somewhat larger fibers. The combined averages of right and left fibers of the 'pneumonia' rats differ very slightly from the combined averages of their controls.

The axis sheath relation in both 'pneumonia' rats and their controls (members of same litter) is practically 40 per cent axis to 60 per cent sheath, a relation found to exist in a group of 15 normal inbred rats 150 days of age, as shown by table 4.

Examination of this small group of animals reveals no significant changes in the sectional area of fibers or in the axis-sheath relation as the result of disease (pneumonia).

Examination of the number of fibers on both right and left sides reveals the fact that the older the animals in both the 'pneumonia' and control groups the less is the number of myelinated fibers. Thus members of litter 7, aged 251 days, have an average of 2251 fibers when right and left nerves of both animals are considered. Members of litter 29, aged 335 days, show in like manner, an average of 2093 fibers, while members of litter 35, aged 454 days show an average of only 1971 fibers.

If, for greater accuracy, the females are omitted, a similar result is obtained; the 251 day rats average 2251 fibers, the 335 day rats 2109 fibers, and the 454 day rats 1997 fibers.

Dunn ('12) observed that old rats (640 days of age) showed a decrease in size of medullated fibers in the ventral roots of the second cervical, and states that her observations were made on albino rats of widely varying weights but not in good health.

Table 3 shows that in the eight animals examined the peroneal fibers of the greatest sectional areas occur in animals 335 days of age, or at the end of the first third of the entire span of the rat's life. In this series animals both younger and older than 335 days have fibers of less sectional areas.

EFFECTS OF ELECTRICAL STIMULATION

In further pursuit of the problem of changes in the myelinated fibers in animals in good health, the experiment was modified and instead of subjecting animals to involuntary exercise the nerve under examination was electrically stimulated in the following manner:

Each animal was etherized, both right and left sciatic nerves were exposed and cut in quick succession. The wound in the left leg was closed, while a small portion of the right peroneal (a branch of the sciatic) was exposed, kept moist with normal salt solution and stimulated for 25 to 30 minutes by being touched at one second intervals by platinum electrodes carrying a weak interrupted current. At the end of this period little or no muscular contraction, following each contact of the electrode, was apparent. The stimulated (right peroneal) nerve and the control (left peroneal) nerve were then removed, fixed in 1 per

cent osmic acid, dehydrated, embedded and sectioned in the usual manner. The stimulated nerve and its control were prepared in the same solutions, thus receiving identically the same technical treatment.

Twelve animals of the same sex and strain and of about the same ages were used in these experiments and the results are here presented in table 4.

TABLE 4

Series 12. All males. Effects (volumetric) of electrical stimulation on the peripheral nerve fibers. (Peroneal nerve.)

NUM-BER	LITTER	AGE	WEIGHT	STRAIN	AU-TOPSY	NUMBER OF FIBERS		AVERAGE SIZE OF FORTY LARGEST FIBERS IN SQUARE MICRA					
								Right peroneal (stimulated)			Left peroneal (intact)		
						Right	Left	Fiber	Axis	Sheath	Fiber	Axis	Sheath
350		162	203	Inbred		2253	2123	96.0	38.3	57.7	101.9	39.2	62.7
363		156	261	Inbred		2080	2143	.68.5	30.2	38.3	94.5	42.8	51.7
369	11	154	243	Inbred	Neg.	2013	2090	84.3	39.2	45.1	105.8	44.7	61.1
370	11	154	245	Inbred	Neg.	2125	2093	91.3	41.6	49.7	90.7	38.9	51.8
373	.	150	230	Inbred	Neg.	1912	1903	107.1	46.7	60.4	95.9	37.9	58.0
374	12	150	223	Inbred	Neg.	1920	1870	94.2	38.8	55.4	99.5	41.7	57.8
375	12	150	218	Inbred	Neg.	2163	2117	99.4	44.9	54.5	95.6	43.2	52.4
376		149	236	Inbred	Neg.	2058	2038	88.3	38.6	49.7	93.6	43.9	49.7
389	16	155	251	Inbred	Neg.	2020	2122	94.9	43.6	51.3	105.7	45.4	60.3
390	16	155	230	Inbred	Neg.	2044	2066	82.1	36.3	45.8	83.6	37.2	46.4
437	33	150	248	Inbred	Neg.	2166	2150	101.8	40.4	61.4	110.6	43.5	67.1
438	33	150	237	Inbred	Neg.	2103	2113	116.2	52.2	64.0	103.3	43.4	59.9
Average ...		153	235			2071	2069	93.7	40.9	52.8	98.4	41.8	56.6
									44%	56%		43%	57%

The age of one animal was 162 days, all the others range between 149 and 155 days, and the group for this purpose may be considered of one age—an average of 153 days. Autopsies made on ten of these animals proved to be negative. Two were not autopsied, but showed no signs of disease. It will be noted that the average number of fibers on the right and left sides is practically identical—right 2071, left 2069—adding further proof of symmetry so far as number of fibers is concerned.

The variability in number is shown by the following determinations.

In making these determinations the formulas given by Davenport ('99) have been used for the standard deviation, σ, the coefficient of variability, C, and the probable errors of these, as well as of the mean, M.

Number of fibers in right peroneal nerve—average of 12 cases. Average age 153 days.

M (mean)	2071	$M_\epsilon =$	$\pm$ 18
σ	96	$\sigma_\epsilon =$	$\pm$ 13
C	4.6	$C_\epsilon =$	$\pm$ 0.60

Number of fibers in left peroneal nerve—average of 12 cases. Average age 153 days.

M (mean)	2069	$M_\epsilon =$	$\pm$ 17
σ	87	$\sigma_\epsilon =$	$\pm$ 11.9
C	4.2	$C_\epsilon =$	$\pm$ 0.54

In examining the sectional area of fibers we note that in 8 of the 12 cases the fibers of the right or stimulated nerve are less in sectional area than those of the left nerve. In one case (No. 363) the difference is large.

Taking the average sectional area of the stimulated fibers we find it to be 93.7 square micra, while the average of the left fibers is 98.4 square micra. The difference here shown between the averages is 4.7 square micra or approximately 5 per cent of the smaller number.

An examination of these measurements by the usual statistical methods gives the following results, table 5.

TABLE 5

	Right peroneal *Stimulated fibers*			Left peroneal *Control fibers*	
M (mean) 93.7 sq. micra	$M_\epsilon =$	$\pm$ 2.3	M (mean) 98.4 sq. micra	$M_\epsilon =$	$\pm$ 1.1
σ	11.8	$\sigma_\epsilon = \pm$ 1.6	σ	5.9	$\sigma_\epsilon = \pm$ 0.8
C	12.6	$C_\epsilon = \pm$ 1.7	C	6.0	$C_\epsilon = \pm$ 0.8

The difference obtained between the stimulated and the control fibers is 4.7 square micra. The probable error of this determination is ± 2.5.

It is seen that the probable error of the determination is more than one-half the difference found between the stimulated and the control fibers. Assuming that the areas of the largest fibers in the nerves of the right and left sides are similar under normal conditions then the difference should be more than three times the probable error in order to be significant.

On further analysis of the data presented in table 4 it is found that when the first six entries are separately considered the average sectional area of the right peroneal fibers is 7.8 square micra less than that of the left, while in the last six entries the average sectional area of the right peroneal fibers is only 1.6 square micra less than that of the left.

Furthermore, in considering the entire group, if No. 363, which presents the greatest deviation from the mean, is omitted, the average sectional area of the right peroneal fibers becomes 96.0 instead of 93.7 and the average sectional area of the left peroneal fibers becomes 98.8 instead of 98.4.

The right peroneal fibers thus average 2.8 square micra instead of 4.7 square micra less in sectional area than the left.

Assuming that the largest fibers of the right and left peroneal nerves are normally similar in sectional area, it would therefore seem that the slight difference in average sectional area noted between the right and left peroneal fibers was not due to the electrical stimulation of the right peroneal fibers, but that this difference is a deviation coming within the limits of normal variation.

The axis sheath relation in this series is nearly the same for both right and left nerves—about 43 per cent axis and 57 per cent sheath.

In a previous paper (Greenman, '13) it was stated that the intact nerve of an operated animal contains fewer medullated fibers than the same nerve from a normal animal of the same age, and that there is a loss in sectional area of fibers of the intact nerve of an operated animal.

From table 4 it will be seen that normal inbred albino rats, of an average age of 153 days, have an average of 2070 fibers in

their peroneal nerves. In a group of seven operated stock albino rats of an average age of 161 days, referred to in the paper cited, the average number of peroneal fibers in the control nerves was 2025.

It is thus apparent that the normal animal, even at a slightly earlier age, has more fibers in its peroneal nerves than were found in the intact (peroneal) nerves of operated animals.

The average sectional area of 10 largest peroneal fibers from normal inbred albino rats of 153 days average age is 108.6 square micra (taken from the original records and not shown in Table 4). In the previous study (Greenman, '13) the average sectional area of 10 largest peroneal fibers from the intact side of operated animals, of 189 days average age, was shown to be 65.7 square micra. Thus it is seen that the normal animal even at a younger age has peroneal fibers of greater sectional area than those found in intact nerves of operated animals.

The present data support therefore the previous conclusion that in the operated rats the size of the fibers in the intact nerve was reduced.

NUMBER OF PERONEAL FIBERS; SIZE OF LARGEST PERONEAL FIBERS IN A NORMAL ANIMAL

The next step was to examine the largest fibers of both right and left peroneal nerves in a series of normal inbred animals and to determine whether symmetry of the right and left sides exists as regards the size of the largest fibers.

Table 6 presents the data bearing upon this point. Here are given the measurements and counts of the fibers in the right and left peroneal nerves of 15 animals of same sex and strain and of about the same age.

Here again it will be noted that the average number of fibers on the right side is practically identical with the average number on the left side—2038 on the right side and 2032 on the left side.

The variability is shown by the following determinations:

Number of fibers in right peroneal nerve—average of 15 cases. Average age 150.9 days.

M (mean) 2038 $\qquad$ $M_\epsilon = \pm 17$

σ $\qquad$ 97 $\qquad$ $\sigma_\epsilon = \pm 11.9$

C $\qquad$ 4.7 $\qquad$ $C_\epsilon = \pm 0.57$

Number of fibers in left peroneal nerve—average of 15 cases. Average age 150.9 days.

M (mean) 2032 $\qquad$ $M_\epsilon = \pm 13$

σ $\qquad$ 80 $\qquad$ $\sigma_\epsilon = \pm 9.9$

C $\qquad$ 3.8 $\qquad$ $C_\epsilon = \pm 0.47$

A comparison of the average sectional areas of the 40 largest fibers of the right and left peroneal nerves shows that in four

TABLE 6

Series 15.   Controls.   Normal number and size of myelinated fibers in peroneal nerve

NUMBER	SEX	LITTER	AGE	STRAIN	WEIGHT	GAIN IN WEIGHT LAST FIFTY DAYS	AUTOPSY	NUMBER OF FIBERS		AVERAGE SIZE OF FORTY LARGEST FIBERS IN SQUARE MICRA					
								Right	Left	Right peroneal			Left peroneal		
										Fiber	Axis	Sheath	Fiber	Axis	Sheath
325	♂	1	150	Inbred	242	34.5		1959	1892	95.3	32.8	62.5	99.9	34.3	65.6
328	♂	2	151	Inbred	210	45.0		2279	2068	113.4	40.7	72.7	103.1	39.0	64.1
340	♂	3	152	Inbred	239	46.5	Neg.	2175	2131	98.3	36.9	61.4	97.8	36.0	61.8
342	♂	4	151	Inbred	274	69.5	Neg.	2078	2158	103.5	38.6	64.9	75.5	29.5	46.0
347	♂	6	147	Inbred	245	55.0	Neg.	2040	2018	77.4	30.0	47.4	70.0	26.5	43.5
380	♂	13	151	Inbred	256	55.5	Neg.	2123	2088	98.7	39.0	59.7	92.1	38.2	53.9
381	♂	13	151	Inbred	283	53.5	Neg.	1985	2029	101.3	43.7	57.6	95.4	38.2	57.2
384	♂	14	153	Inbred	276	33.0	Neg.	1931	1925	99.4	44.0	55.4	81.5	31.8	49.7
385	♂	14	153	Inbred	256	35.0	Neg.	1887	2085	89.7	36.8	52.9	89.5	39.0	50.5
388	♂	15	151	Inbred	238	60.0	Neg.	1967	1985	101.0	41.4	59.6	103.5	41.3	62.2
394	♂	18	150	Inbred	276	53.0	Neg.	2045	2048	100.2	40.3	59.9	97.1	37.2	59.9
395	♂	18	150	Inbred	314	78.0	Neg.	2091	2134	91.4	35.2	56.2	90.6	35.2	55.4
403	♂	21	151	Inbred	266	85.5	Neg.	1982	2003	92.2	35.4	56.8	101.0	41.9	59.1
439	♂	34	151	Inbred	233	43.0	Neg.	1989	1897	97.1	40.5	56.6	82.9	32.2	50.7
443	♂	34	151	Inbred	200	41.5	Neg.	2039	2025	92.6	41.8	50.8	92.9	37.4	55.5
Average			151					2038	2032	96.8	38.5	58.3	91.5	35.8	55.6
											40%	60%		39%	61%

cases the two sides are within 1 per cent of one another; in 8 of the remaining eleven cases the average of the 40 largest fibers is greater on the right side.

The axis sheath relation here is practically 40 per cent axis to 60 per cent sheath.

Taking the 15 cases together, the average size of fibers of the right peroneal is 96.8 square micra, the average of the left 91.5 square micra, the difference being 5.2 square micra, indicating that the largest fibers of the right peroneal nerve are more than 5 per cent greater than those of the left.

Examining these results by statistical methods we obtain the following table 7.

TABLE 7

Right peroneal fibers		Left peroneal fibers	
M (mean) 96.8 sq. micra	$M_\epsilon = \pm 1.3$	M (mean) 91.5 sq. micra	$M_\epsilon = \pm 1.6$
σ 7.6	$\sigma_\epsilon = \pm 0.9$	σ 9.7	$\sigma_\epsilon = \pm 1.1$
C 7.9	$C_\epsilon = \pm 0.9$	C 10.6	$C_\epsilon = \pm 1.3$

The observed difference between the average sectional area of right peroneal fibers and left peroneal fibers as shown by table 7 is 5.2 square micra.

The probable error of this determination is ± 2.1; a little more than one-third of the difference observed.

If, however, the first seven entries of table 6 be considered separately the average of the right peroneal fibers is found to be 7.7 square micra greater than the average of the left peroneal fibers, while in the last eight entries this difference is only 3.1 square micra.   If No. 342, which presents the greatest difference between the right and left fibers, be omitted, then the average of the right peroneal fibers becomes 96.4 instead of 96.8 and the average of the left peroneal fibers becomes 92.7 instead of of 91.6, and the difference becomes 3.7 instead of 5.2 square micra.

From the statistical examination of table 6 and this further analysis of its contained data we may safely assume that the difference here shown between the sectional areas of the largest fibers of the left peroneal nerve and those of the right peroneal

nerve are within the limits of normal variation and that there is practical symmetry between the largest fibers of the right and left peroneal nerves.

Further support of this conclusion is to be furnished by an examination of tables 2, 3, 4 and 6, where it will be found that in the six instances the average of the largest fibers appears greater in three instances on the right side and in three instances on the left side.

INCREASE AND DECREASE IN NUMBER OF FIBERS WITH ADVANCING AGE

In table 8 are brought together certain data from tables 2, 3, 4 and 6 giving the ages of the animals and the number of fibers in the right and left peroneal nerves.

These entries are arranged according to the age of the animal from 147 to 454 days.

They are divided into six age groups, the first group including animals from 147 to 150 days of age, the second, animals from 151 to 154 days of age, the third, animals from 156 to 162 days of age, the fourth, animals 251 days of age, the fifth, animals 335 days of age, and the sixth, animals 454 days of age. The average number of fibers in both right and left peroneal nerves was determined for each age group. The table shows that between 147 and 251 days of age there is a steady increase in the number of peroneal fibers from 2037 in the first age group to 2251 in the fourth age group, roughly about two fibers per day; from 251 days to 454 days represented by two groups of three animals each, the number of fibers decreases to 1971 fibers, the number at 335 days being intermediate. Between 251 days and 335 days there are no records to show when the maximum number of fibers is reached.

SUMMARY OF RESULTS

A preliminary test, in which a gray rat was forced to run continuously for four hours, showed the sectional area of the peroneal fibers to be 20.4 per cent less than in the corresponding fibers of the control animal. The axis-sheath relation showed

3.7 per cent less sheath with a corresponding increase in axis
in the exercised animal.   The animals were of about the same

TABLE 8

Data from tables 2, 3, 4 and 6

NO.	AGE	NUMBER PERONEAL FIBERS		AVERAGE OF RIGHT AND LEFT PERONEAL FIBERS
		Right	Left	
347	147	2040	2018	
376	149	2058	2038	
394	150	2045	2048	
438	150	2103	2113	
395	150	2091	2134	
437	150	2166	2150	
325	150	1959	1892	
375	150	2163	2117	
374	150	1920	1870	
373	150	1912	1903	2037
342	151	2078	2158	
380	151	2123	2088	
381	151	1985	2029	
388	151	1967	1985	
403	151	1982	2003	
439	151	1989	1897	
443	151	2039	2025	
328	151	2179	2068	
340	152	2175	2131	
384	153	1931	1925	
385	153	1887	2085	
370	154	2125	2093	
369	154	2013	2090	2040
363	156	2080	2143	
350	162	2253	2123	2124
352	251	2240	2230	
353	251	2240	2296	2251
424	335	2122	2000	
425	335	2145	2006	
426	335	2122	2165	2093
445	454	2047	1988	
446	454	1973	1863	
447	454	2040	1916	1971

weight, but were not known to be of the same litter or of the same age.

Comparing male 'pneumonia' animals with male controls the total number of right and left peroneal fibers of the diseased animal is in every case less than the total number of right and left peroneal fibers of the control; this difference is 5.8 per cent.

No significant differences were observed between the sectional areas of fibers from 'pneumonia' rats and those from the controls. The axis-sheath relation in both 'pneumonia' rats and their controls is 40 per cent axis to 60 per cent sheath.

In this group of rats from 251 to 454 days of age, the older the animal the fewer the myelinated fibers found in their peroneal nerves. This applies to both 'pneumonia' and control rats. The fibers of the greatest sectional area occur in those animals 335 days of age, the younger and the older animals present fibers of less sectional area.

Data are presented to show that from age 147 days to age 251 days the peroneal nerve gradually acquires more fibers at the rate of about two fibers per day. In two groups of three animals each, aged 335 days and 454 days, respectively, the number of fibers is shown to decrease with advancing age. Between 251 and 335 days of age the rat acquires its largest and its greatest number of peroneal fibers.

In a series of twelve animals in which the right peroneal nerve of each rat was electrically stimulated for thirty minutes the sectional areas of the stimulated nerve fibers were slightly less when compared with those of the left or intact nerve, but this difference may be regarded as insignificant.

Fifteen albino rats were examined to determine the normal size of peroneal fibers of the right and left sides. This examination of the largest fibers showed that there is practical symmetry between the right and left peroneal fibers.

Twelve normal inbred albino rats of 153 days average age have an average of 2070 fibers in their peroneal nerves and the average sectional area of the ten largest fibers of these peroneal nerves is 108.6 square micra.

CONCLUSIONS

In the five series of albino rats here examined the axis-sheath relation varies but slightly. Its range is from 38 per cent axis: 62 per cent sheath to 43 per cent axis: 57 per cent sheath; an average of 40 per cent axis: 60 per cent sheath.

The symmetry as to number of fibers in the right and left peroneal nerves is almost exact, the difference shown in one group of twelve and another of fifteen animals being less than 0.3 per cent.

Pathological conditions like the so-called 'pneumonia' which is more or less acute and occurs during the later period of growth in albino rats, appears to lessen the number of myelinated peroneal fibers, but produces no measurable change upon the sectional area of the largest fibers.

In the examination of fifteen rats measurements show that symmetry as to sectional area of the largest peroneal fibers exists between the fibers of the right and left peroneal nerves.

Electrical stimulation of a peroneal nerve for thirty minutes appears to have no measurable effect upon the sectional area of nerve fibers.

In the data presented there is confirmation of the work of Dunn ('12) that sectional area of myelinated fibers decreases slightly in old age; of the work of Greenman ('13) that the intact nerve of an operated animal loses in both number and sectional area of fibers.

The number of fibers in the peroneal nerve increases with age until age 250 days is reached and begins to decrease at or before 335 days of age. After the first year of life the sectional area of peroneal fibers decreases with advancing age; at 335 days of age this process of reduction has already begun.

LITERATURE

DAVENPORT, C. B. 1899 Statistical methods with special reference to biological variation. John Wiley and Sons, New York.

DUNN, E. H. 1912 The influence of age, sex, weight and relationship upon the number of medullated nerve fibers and on the size of the largest fibers in the ventral root of the second cervical nerve of the albino rat. Jour. Comp. Neur., vol. 22, p. 131.

GREENMAN, M. J. 1913 Studies on the regeneration of the peroneal nerve of the albino rat; number and sectional areas of fibers; area relation of axis to sheath. Jour. Comp. Neur., vol. 23, no. 5, p. 479.

TASHIRO, S. 1912 Carbon dioxide production from nerve fibers when resting and when stimulated; a contribution to the chemical basis of irritability. Amer. Jour. Phys., vol. 32, p. 107.

STUDIES ON REGENERATION IN THE SPINAL CORD[1]

II. THE EFFECT OF REVERSAL OF A PORTION OF THE SPINAL CORD AT THE STAGE OF THE CLOSED NEURAL FOLDS ON THE HEALING OF THE CORD WOUNDS, ON THE POLARITY OF THE ELEMENTS OF THE CORD AND ON THE BEHAVIOR OF FROG EMBRYOS

DAVENPORT HOOKER

Anatomical Laboratory of the School of Medicine, Yale University, New Haven, Connecticut

NINE FIGURES

In the first paper of this series, the attempt was made to analyse the processes leading to the reunion of the spinal cord in frog embryos, following its simple but complete severing just after the closure of the neural folds. The primary purpose of the experiments upon which this paper is based was to test the effect of reversal of a piece of the spinal cord on the healing of such wounds in embryos of the same age. It soon became apparent that this purpose would be overshadowed in importance by the evidence which such experiments would give upon, (1) the nature of the primary responses of the embryos used, (2) the nature of the early nerve development, both within and without the spinal cord, and (3) the effect of the reversal on the polarity of the cord and the neurones. The results detailed below throw light upon some of these points.

Reversal end-for-end of portions of the central nervous system has been carried out by several investigators, notably by Harrison ('98, '03), and by Spemann ('12). As described in his earlier paper, Harrison grafted young frog embryos together by

421

their tail buds and, after from two to six days, cut them apart again, leaving a portion of the tail of one fixed in reversed position to that of the other. From this reversed piece a nearly normal tail regenerated, but the spinal cord of the graft contained "few or no ganglion cells or nerve fibers" and remained in a very rudimentary condition. As described in his later paper, Harrison made a composite embryo of a head and tail in normal orientation, but with a reversed middle section. These embryos were at first helpless, owing to the fact that each component reacted independently of the rest. This, however, was gradually overcome until almost perfect coordination resulted and in some cases the embryos lived for weeks.

Spemann ('12) removed a portion of the medullary plate as soon as it became visible and regrafted it, having turned it end-for-end. As the edges of the wound were carefully apposed, healing *per primum* resulted. The portions of the brain anlagen, which were thus reversed, retained their original polarity. Spemann worked with embryos of Rana, Bombinator and Triton.

In the present experiments, a piece of spinal cord about 1 mm. in length was removed, turned end-for-end and grafted into the space from which it has been taken. In the greater number of cases the anterior cut passed through the extreme caudal portion of the medulla, the posterior cut being 1 mm. caudad to it. For the most part the embryos operated upon were those of Rana sylvatica, but R. palustris and R. pipiens were also used. All these embryos healed well and lived for a considerable period.

EXPERIMENTS

Methods. Before each set of operations was performed, a large number of embryos were freed from the jelly-mass and egg-membranes. The animals to be used in the experiments were carefully chosen from this number. The factors upon which the selection was based were uniformity of size, of stage of development and a healthy appearance. Especial care was taken in matching the normal control animal to each operated specimen. The greater number of the embryos were operated upon in the just closed neural fold stage and were from 2.5 to 3.5 mm. long.

A few averaging 5.5 mm. in length were operated in the stage having fairly well developed tail buds; a few others at a somewhat later stage, in which the tail fin was just becoming visible, when they averaged 6.5 mm. in length. The operations on the 5.5 mm. embryos were as successful as on earlier stages, but it was found impossible to secure good grafts in the 6.5 mm. stage because the voluntary movements of the embryos prevented successful healing. This could have been overcome by the use of anaesthetics, but as the earlier stages fulfilled the requirements of the immediate problem these were not used.

The operations were performed in 0.4 per cent saline or in clean tap-water[2] under a Zeiss binocular microscope. The dorsum of each embryo was cut through in two places, marking out the position and length of the piece to be removed. The cuts severed the skin, cord and the dorsal portion of the myotomes involved, but the notochord was left intact, with a few exceptions. The embryos were then laid on one side and the two vertical incisions joined by cutting horizontally through the skin and myotomes of the upper side. The cord was then carefully separated from the notochord, the myotomes and skin of the opposite side were cut through and the piece freed from the embryo.

If the piece of cord were to be reversed, the excised mass was turned end-for-end, replaced in the gap in the back of the embryo and held *in situ* by piling silver wire about the animal. They were allowed to remain thus until the cut edges of the skin had reunited (fig. 1, A). This takes from fifteen to twenty-five minutes. Of 114 embryos operated, all but 12 were treated in this manner. In these 12 the removed piece was regrafted into the space from which it had been taken without being reversed.

In some of the embryos with reversed pieces of spinal cord, the wound surfaces were very carefully brought into close apposition so that healing *per primum* resulted. This was presumably the condition best adapted to produce a reversal of the polarity of the

---

[2] Clean tap-water has been found to be as satisfactory an operating medium as 0.4 per cent saline (which is isotonic for embryos of this stage) when no very great area of epidermis is to be regenerated. Consequently, it was used in all but the earliest of these experiments.

cord, if such a thing were possible. In the rest no attempt at apposition was made.

Harrison ('03) found difficulty in keeping 'all sylvatica' composites with a reversed middle piece alive for any great length of time. No more trouble was encountered in the course of the
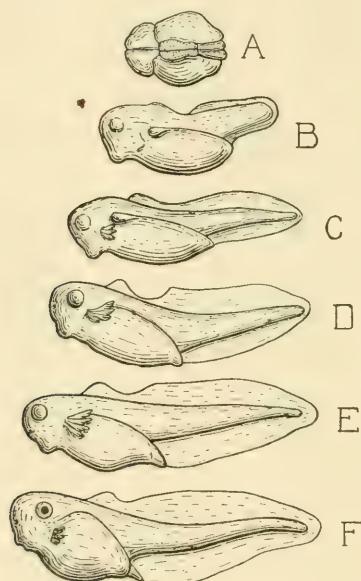
Fig. 1 External form of an embryo in which a portion of the spinal cord was reversed. A, twenty minutes after operation, the stippled area is the piece which has been reversed; B, twenty-four hours after operation; C, two days after operation; D, three days after operation; E, four days after operation; F, seven days after operation. A is a dorsal view, B to F lateral views. These figures show the peculiar spur-like process of the dorsal fin which is characteristic of these embryos. Outlines made with a camera lucida. (Embryo IX, 5.)

present experiments in keeping sylvatica specimens alive than the other species, a circumstance which may be due to the fact that the piece reversed was smaller than in Harrison's experiments. Where the wounds were apposed, few embryos died, but those animals in which fusion was not produced appear to have a limited viability and, though many lived for weeks and became

nearly or quite normal in behavior, a large proportion of them died.

Each embryo, with its normal control, was placed in a separate Syracuse watch glass, was given an individual number and a separate protocol was kept for it.

The embryos were mechanically stimulated at frequent intervals by lightly touching or brushing the surface of the body with a soft human hair, according to the method employed by Coghill ('09 et seq.). This method is very satisfactory, though it must be borne in mind that by poking the embryo even a human hair will penetrate the skin and directly stimulate the myotomes.

The embryos were fixed at intervals of from twenty-four hours to eight days after operation in sublimate acetic, with the exception of those that were to be used for the Bartelmez silver nitrate method, which were fixed in the absolute alcohol and acetic acid mixture. As the embryos developed very rapidly, the eight-day specimens were well advanced.

Serial sections of the embryos were stained with Held's molybdic acid hematoxylin and congo red, Ehrlich's hematoxylin and congo red, erythrosin and toluidin blue or the Bartelmez silver nitrate method.

Wound healing. The method of healing a single, severing cut through the spinal cord has been described in detail in the first paper of this series.[3] A careful study of sections of the embryos with reversed portions of the spinal cord shows that, in the main, the method of healing is the same whether a piece of the cord be reversed or whether it be simply severed, but that in the former case the reversal brings in certain disturbing factors which tend to mask and limit the processes at work. An embryo with the cord severed will, in the majority of cases, re-establish anatomical and functional continuity of the cord whether the wound surfaces heal *per primum* or not, but those embryos in which a piece of the cord was reversed rarely exhibited complete reunion of the cord, though this sometimes oc-

[3] D. Hooker, '15, pp. 471–486.

curred, if primary fusion of the wound surfaces had not taken place. Primary fusion of the severed parts of the embryo always ensued when the wound surfaces were carefully apposed, but such embryos afford little or no evidence of the individual processes leading to the healing of the wound, because no active regenerative changes are visible. Some evidence on this point is afforded, however, by those embryos in which the continuity of the cord was interrupted at either or both ends after the epidermis had regenerated sufficiently to cover the cuts and hold the reversed piece in position. We shall consequently examine these latter embryos rather in detail.

A period of primary repair follows immediately on the operation, whether the cord be severed in one place or in two, even though in the latter case the reversal end-for-end of the tissue between the cuts accompanies the operation. The epidermis plays the major part in this repair, covering over the wound surface so completely that it pushes down between the cut cord ends if they are not fused. The mesenchyme proliferates rapidly and fills up the spaces made by the operation. The open ends of the neural tube become closed by a shifting of the cells already present and new composite myotomes are formed by the fusion of their dorsal and ventral halves.

Sections show that the cephalic cut in nearly all cases passes through the caudal extremity of the medulla. The second cut is found from 1 to 1.5 mm. caudad to it. The caudal (originally cephalic) end of the reversed portion of the cord has usually somewhat greater diameters than the cephalic (originally caudal) end, due to the small part of the medulla attached to it. In many embryos the cut ends of the cord do not lie directly opposite one another, but deviate in various directions from true alignment. The piece reversed is so small that it is difficult to place it exactly in position, but such deviation, unless excessive, does not necessarily hinder the ultimate reunion of the cord.

During this period of primary repair the embryo as a whole continues to grow and the characteristic feature of all frog embryos in which a portion of the dorsum has been reversed begins to appear. This is the peculiar hump on the back which,

thick at first, gradually thins out into a typical dorsal fin, but in reversed orientation (fig. 1, B–F).

In the first paper of this series, it was noted that the epidermis probably plays no rôle in the reunion of the spinal cord, but that further evidence was needed on this point. The close juxtaposition of the epidermis to the cut ends of the cord renders it very difficult to settle this question definitely. In both series of experiments, where the wound edges were not in apposition, a V-shaped invagination of the epidermis occurred between the cord ends in the early stages which, on further development, became a solid fold and was finally withdrawn. In the early stages of this process, the epithelial cells lie in nearly direct contact with the spinal cord ends and are morphologically indistinguishable from the primitive neural cells. The problem is, therefore, the same in both series of experiments. A careful study of many embryos brings out certain facts which, if they do not afford definite proof, at least give strong evidence in favor of the conclusion that the epidermis contributes no elements to the regenerating cord.

An examination of the wound region in an embryo twenty-four hours after operation, of which figure 2 may be considered typical, shows that, even though the epidermal ingrowth lies almost in contact with the cells of the spinal cord, it has a very definite boundary and, except for an occasional cell, its connection with the epidermis covering the body may be clearly seen. In the lower part of the invagination the condition is somewhat more confused owing to the presence of cells from the notochordal sheath, but even here one may differentiate with a fair amount of accuracy between the two types. The presence of mesenchyme cells here and there between the epidermal and neural cells also complicates the problem, but it is usually possible to identify them. The slight space between the cord ends and the interposed tissue seen on the left in figure 2 is too characteristic and constant to be a shrinkage space.

This space between the cord ends and the interposed tissue increases with the growth in length of the embryo, due to the separation of the cord ends from one another and the withdrawal

of the invaginated epithelium.   In this process of withdrawal,
the epidermis maintains no epithelial connection with the cord
by which it could play any rôle in contributing elements to its
regeneration.   The mesenchyme cells slip in between the epi-
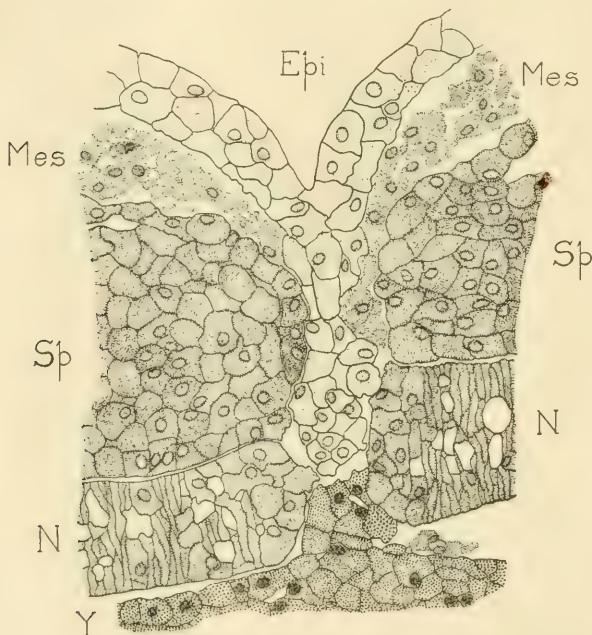
Fig. 2 Condition at site of the wound twenty-four hours after operation.
Epi, epidermis, which has grown down between the cut ends of the spinal cord
and notochord; *Mes*, mesenchyme cells; *Sp*, spinal cord.   Note the slight separa-
tion on the left hand side of the figure from the epidermal ingrowth and the
definite boundary of the neural cells.   *N*, notochord.   Note the large rounded
cells at the cut edges which will proliferate to re-establish its continuity.   *Y*,
mesenchyme cells containing a large amount of yolk.

This drawing is considerably schematized in order that the identity of the
different cells may be more clearly brought out.   As a matter of fact, the epi-
dermal cells, shown lightly stippled in the drawing, are very similar to the
neural cells, shown somewhat darker in the figure.   Both contain a large amount
of yolk, as do the cells of the notochord and the mesenchyme.   The definite con-
tinuity of the epidermal ingrowth and the rounded end of the spinal cord render
identification of the cells possible.   This figure represents the condition follow-
ing the stage of primary repair of the wound.   With further development the
epidermal ingrowth will be withdrawn and the continuity of the two ends of the
cord re-established.   (Embryo VIII, 83.)

dermis and the cord ends as the former is withdrawn, so that the only connections remaining between the two are mesenchymal in nature. The only time at which the epidermis could contribute elements to the spinal cord is in the earliest stage. While it is not possible to definitely state that no such elements are contributed, the continuity of the epithelial tissue and its separation from the cord ends cast strong doubt on such a condition. The mesenchymal elements which become mixed with the developing nerve fibers are cast out at a later stage by the development of ependymal cell fibers and the wandering out of neuroblasts in the same manner as has been described for embryos with severed spinal cord.

In embryos having the cord merely cut in two, the first active regenerative process which is visible is the growth of nerve fibers from both stumps of the cord. From the cephalic stump (fig. 3, I, A) in such cases, there appears a number of rather large nerve fibers which grow toward the caudal stump. These fibers are the descending processes (A, dm) of motor neurones situated within the cord cephalad to the cut. They are the first fibers to appear at the wound surface. From the caudal stump (fig. 3, I, B) there appear shortly after the outgrowth of motor fibers from the cephalic stump, bundles of fibers which are the ascending processes of motor neurones situated caudad to the cut (B, am). At a slightly later time, a number of small nerve fibers appear from the caudal stump growing out from its dorsal portion toward the cephalic stump. These fibers are the ascending processes of the sensory neurones (B, $\mathring{a}s$). No sensory fibers could be identified as such, growing from the cephalic stump. They appear to be very essentially centripetal in their manner of growth.

In embryos having the cord cut in two places, without reversal of the piece between the cuts, a condition represented in figure 3, II obtains. There are four cut surfaces, C, D, E and F, of which C and E are true cephalic stumps of the cord, corresponding to A in number I of the same figure, and D and F true caudal stumps corresponding to B in I. The regenerative conditions found in the wound CD are identical with those in the wound AB in I,

as are those found in the wound *EF*, with the exception of the
fact that the nerve fibers from the latter surface appear at a
slightly later time than those from *CD*. This would be ex-
pected from Coghill's demonstration of the caudally progress-
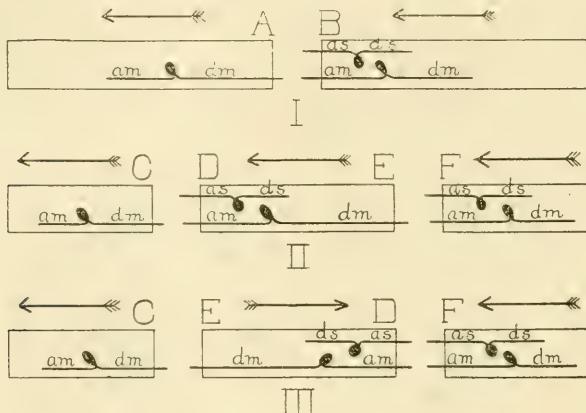
Fig 3 Diagrams showing the nature of the nerve processes growing out
from the cut ends of the spinal cord. The arrows point toward the head, except
in the segment *ED* in III, where it points toward the cephalic end of the re-
versed piece; *am*, ascending motor; *as*, ascending sensory; *dm*, descending motor;
ds, descending sensory processes.

I represents the condition found after a single severing cut through the spinal
cord. From the caudal extremity of the cephalic stump, *A*, only descending
motor processes arise. From the cephalic end of the caudal stump, *B*, both
ascending motor and ascending sensory fibers are developing.

II represents the condition in a spinal cord which has been completely sev-
ered in two places. The wounds *CD* and *EF* each show the same conditions as
the wound *AB* above.

III represents the condition in the spinal cord of an embryo, the middle sec-
tion of which, *ED*, has been reversed end-for-end. The stump *E* shows only
descending motor processes arising from it, while the stump *D* has both sen-
sory and motor fibers. A comparison of figures II and III shows that by the
reversal of the position of the middle segment no change has been brought about
in the nature of the processes which arise from the surfaces *D* and *E*.

ing differentiation of the neurones of the cord. In both of these
wounds the motor fibers from the cephalic surfaces are the first
to appear, those arising from *E* making their appearance some-
what later than those from *C*, but before the sensory fibers have
begun to grow out from *D*. The motor fibers appear in the

order C, D, E, F. They are followed by the appearance of sensory fibers, first from D and later from F.

If the segment DE is reversed in position, as shown in figure 3, III, the two originally cephalic stumps, C and E, are brought into apposition, as are the two originally caudal stumps, D and F. Two possibilities for the outgrowth of nerve fibers from the stumps in this position present themselves. Either (1) the original polarity of the neurones and the fibers, being an inherent function of the cells themselves, will be maintained in their reversed position and the surfaces C, D, E and F will exhibit the same kind of fibers as they would have done in their original position, or (2) the polarity of the neurones and their fibers, if a function of their position, would be reversed with the reversal of their position, so that D would show only motor fibers growing from it and E both motor and sensory.

Serial sections of a considerable number of embryos in this stage have been available and they demonstrate that the first of these possibilities is the one which actually occurs. The position of the cord segment is reversed but the polarity of the neurones within that segment is unaltered. The surface of the stump C exhibits only nerve fibers which are the descending processes of motor neurones. The surface D presents fibers which from their morphological character and from their position must be ascending processes of both motor and sensory neurones. Such a condition is also exhibited by the wound surface F, while E exhibits only motor fibers.

One most noticeable characteristic of the further development of these nerve fibers is that they show a marked tendency to avoid entering the wound surface opposite them. While in the simple cord severing experiments, it was quite rare to find the ascending branches of the sensory neurones from the caudal stump B wandering away from and not entering the cephalic stump A, in the embryos with a reversed piece of the cord, it is quite rare to find any sensory fibers from F entering D or *vice versa*. The motor fibers also exhibit this antagonism, but to a less degree, so that at least partial motor union between the two adjacent surfaces is fairly common. It is this avoidance of the

opposite wound surface chiefly on the part of the sensory fibers which causes the failure of so many of the embryos with primarily unfused wounds to re-establish continuity of the cord and, indeed, to live. This apparent antagonism between 'like' wound surfaces has been noted by many investigators who have worked on the reversal of the position (and polarity) of parts of organisms. That it exists in those embryos in which the wounds are caused to heal *per primum* by close approximation of the cuts is without doubt, but the very fact of fusion prevents its expression in so marked a manner as is permitted by the separation of the cord ends in the embryos under discussion. The fact that in a very few cases the late stages of complete reunion have been obtained in these embryos, does not militate against the effectiveness of the antagonism as a factor which oppose such re-establishment of the continuity of the cord, as, in all these embryos, the healing is not as clean cut as in those with unreversed cords.

In those embryos in which complete healing is being effected the same stages as those found in the re-establishment of continuity after simple severing (outgrowth of ependymal fibers, wandering out of cells into the fiber mass and elongation of the canalis centralis) are to be found, though in somewhat more fragmentary form in the majority of cases. A very few embryos show complete healing and these differ in no essential respect from the final stages observed after a single severing out of the cord beyond the presence of a number of irregularly situated nerve fibers which run out from the cord into the surrounding mesenchyme to end there, apparently blindly.

The reversed middle piece. Whether the reversed piece has fused with the normal cord and medulla at operation, has healed at one or both ends by the active regenerative processes indicated above or has failed to establish union at either end, it still possesses certain characteristics which make it readily identifiable. The cephalic cut, it will be remembered, passed through the caudal end of the medulla, leaving a part of the cavity with the piece to be reversed. This cephalic end, now directed caudad, shows the fragment of medullary ventricle as a small, usually almost spherical cavity (figs. 4, 5, 6) in all but two cases.

Where the continuity of the cord was restored by either active or *per primum* healing, this cavity opens into the canalis centralis of the caudal end of the cord. The original, rather wide funnel-shaped cut edge of the ventricle has been rounded over, chiefly by the curling downward of the very thin dorsal wall, a portion of the inferior medullary velum. Where the part of the medullary ventricle reversed was considerable, the rounding-
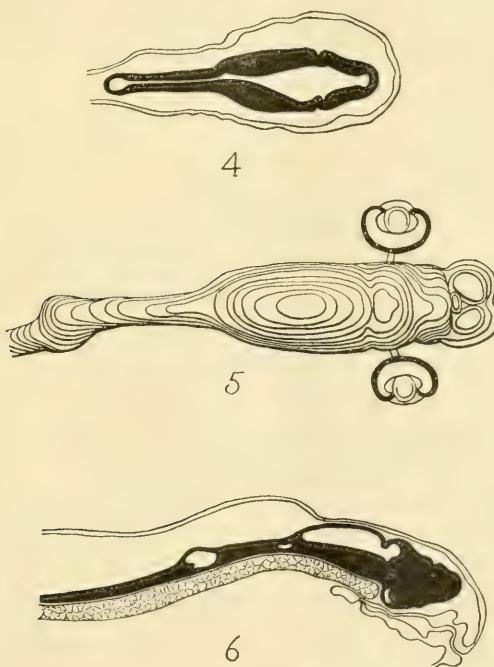
Fig 4 Frontal section through a portion of an embryo, the spinal cord of which was reversed. At the left hand side of the figure the remnant of the medullary ventricle is visible and in the middle of the figure the slight unevenness of the central canal marks the point of fusion of the cephalic cut. It will be noted that the walls of the medullary ventricle have become narrowed.

Fig 5 Graphic reconstruction of the central nervous system of the same embryo. (Embryo IX, 9.)

Fig. 6 Sagittal section through an embryo, a portion of the spinal cord of which has been reversed. A transposed portion of the medullary ventricle is clearly to be seen. (Embryo IX, 13.)

over process has been less abrupt and a larger, more fusiform cavity results (figs. 7, 8).

In one case, a piece of the notochord which was cut at operation became interposed between the caudal wound surfaces (fig. 9). Healing was *per primum* and the end of the notochordal fragment became covered over by ependymal cells, thus projecting into the canalis centralis. As the fragment was in continuity with the rest of the notochord, the ventral fiber tracts were shifted laterally and upward to avoid the obstacle. Function, in spite of the abnormal distribution of the fibers, became perfectly normal.

Where the fusion of the cephalic wound has been complete, the canalis centralis of the originally caudal end of the reversed piece of the cord, now fused with the caudal end of the medulla, has undergone enlargement in a few cases. As a consequence, the medullary ventricle passes over into the canalis centralis of the spinal cord by a gradually tapering funnel. This is the normal condition of course, but in these operated embryos the wall of the funnel is formed in part from the ependymal cells of a portion of the canalis centralis normally situated a millimeter or more away. The point of fusion is noticeable in many of the specimens as a more or less pronounced notch in the ventricular wall, though in others the fusion is so perfect as to make it impossible to detect the line of healing. In other cases, however, it seems that the caudal extremity of the medullary ventricle has undergone a narrowing to produce this funnel (fig. 4), and that the canalis centralis of the reversed piece of the spinal cord has been unaltered in the process. In those cases where fusion or healing did not occur at the cephalic cut, the caudal end of the medulla is rounded over and closed. In some of these cases the ventricle is much enlarged.

Effect of reversal of position upon the polarity of the cord. In the operative procedure described above, a piece of the spinal cord has been removed from its normal position, turned end-for-end and been grafted into the space from which it was removed. Its position was therefore reversed. The evidence given demonstrates that the neurones contained within that reversed piece

continued to develop in their normal orientation to that piece and that the transposed portion of the medullary ventricle remained, with but minor changes, normal.   During the early stages of development and up to a time after the healing has been completed, there is not a single sign that the polarity of the cord has been reversed.
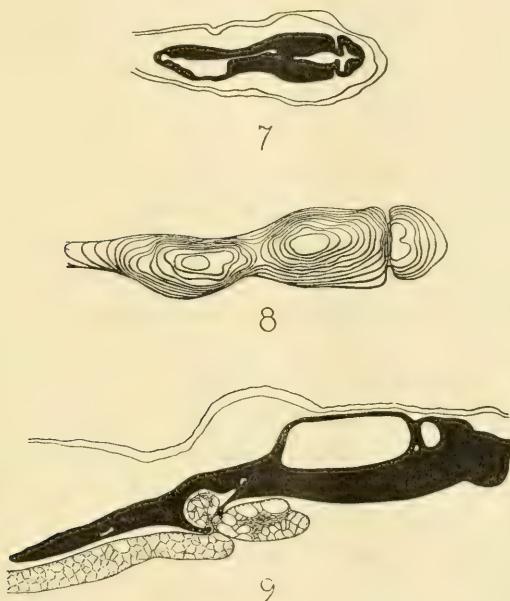
Fig. 7   Frontal section through an embryo in which a relatively large part of the medullary ventricle (seen at the left) was reversed.

Fig. 8   Graphic reconstruction of the same embryo.   (Embryo IX, 12.)

Fig. 9   Sagittal section through the central nervous system and notochord of an embryo in which a small portion of the spinal cord was reversed.   The right hand piece of the notochord marks out the length of the segment reversed. It will be noted that the central canal of the spinal cord has apparently enlarged at the point of fusion with the medulla anteriorly.   Posteriorly a piece of the notochord became inserted between the wound surfaces, necessitating the divergence of the developing nerve fibers from the normal course.   In spite of the cavity in the spinal cord in which this piece of notochord lies, a return to normal function was obtained.   This section is from the embryo shown in figure 1. (Embryo IX, 5.)

In those embryos in which healing has not occurred no sign of a reversal of polarity ever presents itself. For any evidence that later adaptation produces anything similar to reversal of polarity in the older stages of the healed embryos, we must turn to a consideration of their behavior.

Responses of embryos with primarily fused cords to tactile stimulation. As previously noted, the embryos were stimulated with a human hair according to the method of Coghill. Embryos in which a portion of the spinal cord had been reversed and in which primary fusion had taken place began to exhibit responses to tactile stimulation from twenty-four to thirty-six hours after operation. In the case of those embryos operated when from 2.5 to 3.5 mm. in length reactions to stimulation appeared in the latter part of this period in most cases, while those operated at a later stage, averaging 5.5 mm. in length, usually reacted twenty-four hours after operation. In every case the first reaction to appear was a decided bending of the head toward the side stimulated. But a single response followed each stimulation. Inasmuch as Coghill ('09) has described this type of reaction as occurring only occasionally and very irregularly in Amblystoma, a large series of normal frog embryos were carefully stimulated with a human hair in order to obtain information in regard to the frequency of its occurrence in that animal. The results of these experiments demonstrated quite conclusively that in the frog embryo a contraction of the myotomes of the same side as that which receives the stimulation is the first normal type of reaction to tactile stimuli. It is of course evident that direct stimulation of the myotomes would result in a similar type of response. Great care was therefore taken to be sure that the pressure of the hair upon the embryo was not sufficient to directly stimulate the myotomes. To render control of this point more satisfactory all stimulation was performed while the embryo was being watched with a Zeiss binocular microscope. It was found that by gently touching the surface of the embryo with a slow stroke-like movement of the hair at a point just ventral to the center of the myotomes, without the slightest indentation of the skin of the tadpole having

been caused by the pressure of the hair upon it, this type of response could be almost unfailingly obtained. The constant reappearance of this type of response lends further evidence in favor of the view that a single quick bending of the head toward the side stimulated is the most primitive reaction to tactile stimulation in the frog embryo.

This type of response is quickly followed by a typical 'avoiding' reaction. This reaction makes its appearance within two to three hours after the beginning of sensitivity to tactile stimulation of the embryo and lasts for a varying period.

Embryos from thirty-six to forty-eight hours after operation almost uniformly exhibit a double C reaction. The term 'double C' reaction has been applied to that form of response in which the embryo on stimulation contracts into an arc, straightens out and contracts into a similar arc on the opposite side. This constitutes the complete reaction to a single stimulation. This is true even of those embryos which do not begin to exhibit any response until after the first thirty hours. These embryos which are late in beginning their reaction appear to hasten through the earlier stages so that they tend to become uniform in their response at this later period. It was noted that at the very beginning of this type of response there seemed to be a tendency on the part of many of the embryos to contract first on the side stimulated, but there is not sufficient evidence to prove that the first contraction toward the side stimulated is any more decidedly frequent in appearance than a first contraction away from the side stimulated. During the beginning of the appearance of this type of reaction the two contractions toward opposite sides of the body constituted the entire response, but within a very short time, frequently not more than half an hour afterwards, the embryos exhibited a series of these double C reactions to each stimulation.

This type of reaction lasts for a relatively long time, from six to twelve hours, and it is followed by a typical S or sinuous reaction. During the latter part of the double C reaction period, at a time which apparently bears no relation to the beginning of the appearance of the S reaction, the embryos exhibit spon-

taneous movement for the first time. This is from two and a
half to three days after operation. The S reaction, which is of
course the most primitive manifestation of the swimming move-
ment, rapidly passes over into a period in which active locomo-
tion is produced. In these embryos with the reversed spinal
cord the first movements producing locomotion are ill coor-
dinated and the consequent movement of the embryos is far
from normal. As a rule spontaneous movement on the part
of these operated embryos takes the form of the double C reac-
tion for a considerable period after locomotion may be pro-
duced as a result of stimulation. From a careful study of the
movements of these embryos with reversed middle piece it is
evident that there is little or no coordination between the por-
tions and that locomotion is produced by the activity of the
central (reversed) portion of the body which drags the rest of the
organism into activity. It is true that, during these movements
which produce locomotion, both head and tail portions of the
embryos do move by the contraction of the myotomes situated
in them, but there is every indication that the stimulation of
these myotomes in the head and tail is brought about directly
through the pull of the skin over them which is caused by the
movements of the middle piece. In a previous paper ('11) I
demonstrated that very slight tension on the skin of an embryo
produced by pressure at a distant point is capable of mechani-
cally stimulating the myotomes to activity. The cause of the
movements in these embryos is apparently identical with that
described in Paper I of this series.

From this time on the embryos show a steadily increasing
sensitivity to tactile stimulation and a steadily increasing
amount of coordination in the responses. As soon as even the
most primitive type of coordination was exhibited in the reac-
tions to tactile stimulation, the embryos began to show spon-
taneous swimming movements, which with the passage of time
gradually improved until five to six days after operation many
of the embryos were almost, if not entirely, normal in their
movements. Indeed in several cases it was only possible to
differentiate the operated embryos from their normal controls

by the peculiar spur remaining from the dorsal fin upon the back (fig. 1, F).

Responses of embryos with unfused spinal cords to tactile stimulation. Embryos in which the middle section of the spinal cord has been reversed and in which the wounds have not been permitted to fuse begin to respond to tactile stimulation approximately at the same time as those in which primary fusion of the wound surfaces has taken place. Those operated in the open neural fold stage seldom show any reaction to tactile stimulation until about thirty-six hours after operation, while those which were operated in the beginning tail bud stage begin to respond about twelve hours earlier. This is undoubtedly due to the greater differentiation of the nervous system at the time of operation in the latter. The majority of the stimulations of these embryos was carefully watched under the binocular, as the regions are small and consequently their movements difficult to perceive accurately with the naked eye. In these embryos, of course, there is complete anatomical separation at the sites of the two wounds in the earlier stages. Care was taken that the myotomes were not directly stimulated.

The first response to tactile stimulation is exhibited by the middle piece, irrespective of the age at which the embryos were operated upon. The earliest visible response is a slight twitching of the myotomes on the same side as that which receives the stimulation. Within two hours after this, these embryos exhibit a marked contraction of the middle piece into a well defined arc, the concavity of which is toward the side stimulated. Attempts were made to carefully stimulate only minute areas of the middle piece, but contractions of the entire segment resulted in every case. It was impossible to demonstrate any greater sensitivity to tactile stimulation of one end of the reversed piece over the other, or of any tendency on the part of the myotomes of either end to contract more vigorously than those of the other. The reversed middle piece reacted throughout as a unit. If the cut passed through the caudal extremity of the medulla, the head remained inert for some time after the middle piece had begun to respond. If the cut, on the other hand,

passed through the cord just behind the medulla, the head exhibited a single reaction toward the side stimulated. The tail never showed responses to tactile stimulation until a later time than the middle piece. This type of reaction to stimulation on the part of the middle piece lasted for several hours.

The second phase of reaction to stimulation on the part of the embryos was marked by the appearance of contractions of the middle piece into an arc, the concavity of which was away from the side stimulated. This is of course a typical avoiding reaction. The entire middle piece again responded to stimulation as a unit. At a time which practically coincided with the beginning avoiding reaction in the middle piece, sometimes slightly preceding it, but more usually following it, the head began to exhibit a single movement toward the side stimulated. The myotomes involved were those situated at the extreme caudal end of the head region. The tail in the majority of cases remained unresponsive to stimulation during this second phase of reaction, though in a few cases, just before the beginning of the third phase it also exhibited a single contraction toward the side stimulated.

The third phase of reaction is marked by the passage of the middle piece from the simple avoiding reaction to the double C type. Usually at a time which precedes this by not more than one to two hours, the head has begun to exhibit an avoiding reaction and, almost coincident with its appearance, the tail begins to respond by a single movement toward the side stimulated. The tail portion passes through this period of primary response toward the side stimulated very rapidly and soon enters upon a period in which it gives a typical avoiding reaction. The characteristics of the third stage may therefore be said to be as follows: (a), head gives an avoiding reaction; (b), middle piece exhibits double C reaction; (c), tail passes from a brief stage of reaction toward the side stimulated to one in which a typical avoiding reaction is shown.

The fourth stage of reaction to stimulation is characterized by the appearance of the S reaction in the middle piece and of the double C reaction in both the head and tail segments. At this

stage locomotion of the embryo as a whole will result from continued stimulation of the middle piece. The locomotion thus produced is in every way similar to that exhibited by those embryos in which the wounds have been primarily fused, but appears at a slightly later time. An explanation for the apparent delay in the appearance of the S reaction in the middle piece of those embryos in which the cord wounds were not permitted to fuse is not entirely clear, unless it be that the isolation of the middle segment from the head and tail and the consequent absence of the support which a continuous skin over the embryo gives to it does not enable the myotomes to express their contraction to as marked a degree.

From this time on, the development of locomotion in response to stimulation proceeds slowly and is accompanied by the active regenerative processes which go forward in the embryo. Complete return to a normal condition of coordinated locomotion was seen only in two or three embryos in which practically complete reunion of the spinal cord had taken place.

Spontaneous movement appears in the middle piece as soon as the double C reaction makes its appearance and is followed by voluntary movements of the head when it also passes into the double C phase. The tail is the last portion of the embryo to exhibit spontaneous movement.

Correlation between the phase of reaction and stage of regeneration. From the work on embryos in which the spinal cord has been simply severed, it is apparent that the embryo is capable of performing swimming movements in response to stimulation before there is any nervous connection between the two ends of the cord and that a very simple type of voluntary swimming movement may arise before these connections have been established. It is further to be noted that apparently the only rôle played by the nervous connections in the embryo is that of coordination in the later phases of the swimming movement. Exactly the same conditions are met with in all embryos in which a portion of the spinal cord has been reversed, whether the operation has been followed by the complete fusion of the wound surfaces or not. It has been noted in these experiments

that, not only may a very primitive type of swimming movement be developed, but that a type which is apparently fairly well coordinated may be exhibited by embryos in which no nervous connection is present between the cut ends of the spinal cord.   This was noticed particularly in the case of several embryos in which the reversed middle piece healed in a somewhat oblique position to the long axis of the embryo.   These embryos, though they lived for a considerable time, long after some sort of nervous connection had been established in the majority of other specimens, did not exhibit any connections of a nervous nature between the ends of the reversed piece of the central nervous system and that contained in the head and tail region. Nevertheless, the S reaction in these embryos gave place to the usual uncoordinated swimming movement in due course, which continued to exhibit all the signs of progressive coordination which had been previously supposed to accompany the establishment of nervous connections between the severed cord ends. These embryos of course never swam in a perfectly normal manner, but they developed the ability to move over relatively long distances.   Their movements showed a considerable degree of synchrony between the different nervously isolated portions of the body and the head and tail regions took part in the movements.   It is of course certain that there is no nervous connection through the skin in any such sense as Wintrebert ('04) supposed.   Careful examination demonstrated that there are no nervous connections between the two regions of the body, beyond the possibility of the innervation of a pair of myotomes on either side of the cut surfaces.   That this possibility is not a probability is demonstrated by the fact that such nervous connections have never been observed in these embryos and that the heavy mass of notochordal connective tissue which grew out from the injured notochord in these specimens completely isolated the cut ends of the cord in the middle piece from the other portions of the body.   It is much more probable that the tension on the skin of the embryo caused by the movement of the middle piece has brought about a direct mechanical stimulation of the myotomes of the head and tail regions which

has excited them to contraction. This contraction may have acted as a proprioceptive stimulus to the nervous system of the head and tail region which caused in turn a contraction of the opposite side of the body.

It is therefore to be noted that apparently the activity of the middle piece is responsible for the early movements of embryos in which this middle piece has been reversed. In a few individuals the middle piece sloughed out immediately after operation and these embryos continued their development with this wide gap in the back. Such embryos never exhibited even an approach to a swimming reaction. They were never able to produce locomotion over the most limited distances. Indeed it can be said that the middle piece is the only one which exhibits a true S reaction.

On the other hand, it must be remarked that for a normal swimming movement some sort of nervous connection between the cut wound ends is essential. From a careful study of the stages of regeneration in the spinal cord in correlation with the type of swimming movement exhibited, it seems that motor connections are alone essential for a normal swimming movement. It is further apparent that the ascending motor processes, which are the ones which bridge the caudal cut, are capable of acting as typical descending processes and it is evident that motor stimuli from the middle piece are transmitted along these fibers to the tail portion of the embryo. Conversely, it is also apparent that the descending processes growing out from the cephalic portion of the piece may also change their functions and transmit stimuli to the head region of the embryo. In this sense there is a reversal of the polarity of the neural elements contained within the reversed portion of the cord. On the other hand, there is considerable question as to the specificity of these two types of processes.

The establishment of sensory connections between the isolated portions of the cord is long delayed in the embryos under discussion and seems to play little or no rôle in the development of the swimming movement.

DISCUSSION

From the foregoing résumé of the details of the experiments, it is evident that when a portion of the spinal cord taken from the cervical or upper thoracic region of the frog embryo is removed, turned end-for-end and grafted into position, the piece as a whole retains its original polarity. This is what one would expect from the results obtained by Spemann ('12). The fact that the reversal of position does not affect the original polarity of the piece reversed is amply demonstrated by the persistence of the medullary cavity at the originally cephalic end of the middle piece. Furthermore, the manner in which the developing nerves grow out from the cut ends of the reversed middle piece demonstrates that in the beginning at least the reversal of position does not affect the polarity of the elements contained within the piece reversed. The nerve processes arising from these ends are exactly the same in kind as those which would have arisen if the piece had remained in its original position. This fact complicates the ensuing attempts to re-establish the continuity of the cord. After simple severing of the cord, the descending processes of the motor neurones situated at the various levels of the cord began their development in normal orientation to the cord as a whole. There is in reality no true regeneration of the individual elements, inasmuch as the operations are carried out before the time when the nerves normally begin to develop. In consequence of this fact, these processes are subjected to no other abnormal condition than the necessity of traversing an area filled wtih connective tissue. The same is true as regards the ascending processes of the sensory neurones. In the embryos under discussion, on the other hand, the normal relationship between the direction of growth of all the processes and the antero-posterior axis of the embryos has been completely upset so that the nerve fibers which were originally descending processes grow in an ascending direction and *vice versa*. As we have seen, this brings together at the cephalic wound surface a series of nerve fibers growing in opposite directions which are all descending processes and at the caudal wound a number growing

in both directions which are ascending processes. As we have
noted there is apparently an antagonism between these 'like'
surfaces, demonstrated by the marked tendency on the part
of the nerve fibers to avoid entering the opposite wound surface
in those embryos with open wounds. In spite of this fact some
of these fibers do enter the opposite cut surface and we may be
very certain, in the case of those embryos in which *per primum*
healing took place, that all of the fibers from the reversed middle
piece grew in an abnormal direction.

It is of course doubtful whether there is any real specificity
of ascending and descending processes and the physiological
result obtained certainly demonstrates that a considerable de-
gree of adaptation has taken place here, in that the descending
processes must certainly function as ascending processes and
vice versa. In this sense therefore, we must conclude that
there is a reversal in the polarity of the elements contained
within the reversed piece of the spinal cord, though whether this
reversal in polarity includes anatomical reversal of the cells
themselves is very doubtful. It is much more probable that
only the direction in which the stimuli travel along the processes
is the reverse of its usual course.

Not only do the nerve cells situated within the reversed piece
of the spinal cord show a considerable degree of adaptability, but
the piece as a whole adapts itself in a remarkable degree to its
new environment. This is shown at the anterior wound surface
in several embryos. Here we note that the canalis centralis of
the reversed piece of the spinal cord (originally situated a milli-
meter or more away from its present position) has become
enlarged to form a rather typical funnel shaped outlet for the
medulla, or the medullary ventricle has become contracted for
the same purpose. In the first case, this has been accomplished
by the thinning out of the dorsal portion of the spinal cord to
such an extent that, in one or more cases, the point of union
between the cord and the inferior medullary velum is indistin-
guishable. In the latter case, the more usual one, the walls of
the medullary ventricle have become thickened.

A more extreme example of the capability for adaptation on
the part of the spinal cord is demonstrated in another embryo
(fig. 9) in which a portion of the notochord became inserted be-
tween the posterior wound edges at operation. In this case,
we have a resulting fistula into the canalis centralis occupied
by the end of the piece of notochord, but the embryo appar-
ently suffered no ill effects from the consequent diversion of the
nerve tracts from their normal course. In fact, this embryo
was one of the best in point of executing the various movements
and was only distinguishable from normals by the spur of the
dorsal fin. In spite of the large cavity in the ventral surface of
the spinal cord, the connections between the original caudal end
of the reversed piece and the medulla were apparently completely
re-established. This necessitates, of course, an increase in the
number of fibers which pass laterally along the side of the cord
and which must have bridged an open gap to re-establish the
continuity.

The results of the observations on the nature of the primary
responses in these embryos with a reversed middle piece demon-
strates certain very important facts in regard to their origin. It
is to be noted that each portion of the embryo goes through a
graded series of responses to tactile stimulation which is as
follows: (1), contraction of the myotomes on the same side as
that stimulated, giving a bending of the body toward the side
stimulated; (2), a contraction of the myotomes on the opposite
side from that which receives the stimulation, giving an avoiding
reaction; (3), an alternate contraction of the myotomes on oppo-
site sides of the body, giving a double C reaction; (4), a primi-
tive swimming movement. Furthermore it is to be noted that
the middle segment of the body, which has been reversed in
these experiments is the region which actually determines the
locomotion of the animal as a whole and it is in this region that
this succession of responses to tactile stimulation is best to be
observed. In the head region we note that the reaction toward
the side stimulated and the avoiding reaction are of the same
nature as in the other parts of the body. The double C reac-
tion consists of a side-to-side swaying of the head, for owing to

its shortness there is not sufficient length to give a marked arc. For the same reason there is no true S reaction in the head region. The tail region exhibits all the above phases except that the S reaction is very much later in making its appearance than is the case with the middle section of the body.

The reactions in the middle piece of the body can not be localized in any portion of it, either as regards the sensitivity of the receptors or the functioning powers of the effectors. We must therefore consider that this region of the frog embryo, about one mm. in length and extending from the caudal end of the medulla backward, is a unit not only in its reaction to stimuli, but also as regards the development of the primary nervous connections within the cord. It would seem then that in the frog embryo there is a larger area in which the primary nervous connections are developed simultaneously than is found to be the case in Amblystoma (Coghill).

SUMMARY

1. Reversed portions of the spinal cord heal *per primum* when the edges of the cut have been carefully apposed. When they have not been apposed, the wounds may heal according to the same principles and by passing through the same stages as do wounds caused by simple severing of the cord.

2. No healing has been obtained in embryos of a later stage than that having a fairly well developed tail bud.

3. The reversed portion of the spinal cord retains its original polarity.

4. The neurones begin their development in normal orientation to the reversed piece, but the direction of the transmission of stimuli is apparently reversed at a later time. Their morphological polarity is therefore unaffected by their reversal in position but adaptation causes a subsequent reversal of the functional polarity.

5. Embryos in which a portion of the spinal cord has been reversed are not as a rule as viable as those in which the cord was simply severed.

6. A marked tendency on the part of the nerve fibers to avoid entering the opposite wound surface of the spinal cord is noticeable in these embryos.

7. These embryos render an analysis of the primary responses to tactile stimulation possible. The middle piece is the first portion of the body to respond to tactile stimulation and it reacts as a unit.

8. The first type of response to tactile stimulation in these embryos is a single contraction of the myotomes on the same side as that which receives the stimulation.

9. In spite of the reversal of a portion of the cord the responses to tactile stimulation of these embryos are normal in character, but are somewhat tardy in their appearance. This is particularly true of those movements which are coordinated.

10. Embryos with a nervously isolated reversed middle piece are capable of developing a fairly coordinated swimming movement by means of the tension of the skin over the body.

LITERATURE CITED

COGHILL, G. E. 1900 The reaction to tactile stimuli and the development of the swimming movement in embryos of Diemyctylus torosus, Eschsholtz. Jour. Comp. Neur., vol. 19, pp. 83–195.

1913 The primary ventral roots and somatic motor column of Amblystoma. Jour. Comp. Neur., vol. 23, pp. 121–143.

1914 Correlated anatomical and physiological studies of the growth of the nervous system of Amphibia. I. The afferent system of the trunk of Amblystoma. Jour. Comp. Neur., vol. 24, pp. 161–233.

1916 II. The afferent system of the head of Amblystoma. Jour. Comp. Neur., vol. 26, pp. 247–340.

HARRISON, ROSS G. 1893 The growth and regeneration of the tail of the frog larva. Arch. f. Entw.-mech., Bd. 7, S. 430–485.

1903 Experimentelle Untersuchungen über die Entwicklung der Sinnesorgane der Seitenlinie bei den Amphibien. Arch. f. mikr. Anat., Bd. 68, S. 35–149.

HERRICK, C. J. AND COGHILL, G. E. 1915 Development of reflex mechanisms in Amblystoma. Jour. Comp. Neur., vol. 25, pp. 65–85.

HOOKER, DAVENPORT 1911 The development and function of voluntary and cardiac muscle in embryos without nerves. Jour. Exp. Zoöl., vol. 11, pp. 159–186.

1915 Studies on regeneration in the spinal cord. I. An analysis of the processes leading to its reunion after it has been completely severed in frog embryos at the stage of closed neural folds. Jour. Comp. Neur., vol. 25, pp. 469–495.

1916 Some results from reversing a portion of the spinal cord end-for-end in frog embryos. Anat. Rec., vol. 10, pp. 198–199.

SPEMANN, H. 1912 Über die Entwicklung umgedrehter Hirnteile bei Amphibienembryonen. Zool. Jahrb., Suppl. Bd. 15[3], S. 1–48.

WINTREBERT, P. 1904 Sur l'éxistence d'une irritabilité excitomotrice primitive, indépendante des voies nerveuses chez les embryons ciliés de Batraciens. Comp. rend. Soc. Biol., t. 57, pp. 645–647.

GLYCOGEN IN THE NERVOUS SYSTEM OF VERTEBRATES

SIMON H. GAGE

Cornell University, Ithaca, N. Y.

TEN FIGURES (ONE PLATE)

HISTORICAL SUMMARY

The brilliant series of investigations by Claude Bernard on sugar in the blood which culminated in his discovery and isolation of glycogen in 1857, may justly be characterized as epoch-making for the understanding of animal physiology, and a proper correlation of the physiology of animals and plants, and their fundamental similarity.

In this series of investigations, Bernard was disturbed and puzzled by not finding at any period of the life of animals glycogen in the nervous system. He missed it also in other organs and tissues, but all the gaps were filled up by one investigator or another until in 1904 the glycogenic function had been demonstrated in some stage of development for all organs and tissues except the nervous system.

This is what Bernard himself says ('59) and practically repeats in all of his published papers and books upon Glycogen:

A aucune époque de l'évolution organique, je n'ai pu constater la matière glycogène dans les tissue nerveux. J'ai traité, soit par la coction, soit par divers autres moyens précedemment indiqués, le cerveau, la moelle épinière . . . . chez des foetus d'homme, de veau, de mouton, de lapin, et à aucune age je n'ai pu y constater la moindre trace de matière glycogène.

Barfurth ('85, p. 299) referring to what Bernard says concerning glycogen in the nervous system of vertebrate embryos, says: "Ich kan diese Angeben lediglich bestätigen."

451

In Pflüger's book on glycogen (2nd edition, '05, pp. 159–160) is the statement with reference to the nervous system of the adult; that Pavy (1881 had reported the presence of glycogen in a normal adult brain analyzed by him, and that Cramer ('80) had found traces in the brain of a person dead of diabetes. On the other hand Barfurth ('85, p. 297) and others, reporting the analyses for the normal adult brains of rabbits and dogs, asserted that no glycogen was found by them.

Pflüger says further on with reference to glycogen in the nervous system in embryos:

Auch im Embryonalzustand ist bisher kein Glykogen in der sich bildenden Nervensubstanz aufgefunden worden, was bereits Claude Bernard untersucht. In neuester Zeit haben G. Fichera ('04) sowohl als E. Gierke ('05) das Nervensystem auf Glycogen untersucht und nur negative Ergebnisse gemeldet.

In 1904 I was fortunate enough to discover the presence of glycogen in the nerve cells of the central nervous system of Amphioxus, and began then a systematic investigation of the nervous system of vertebrates, believing that in some period of development glycogen would be found in the nervous system of each form in the ascending series up to and including man. The following paper is a summarized statement of the results of the work up to the present. For the more extended study, forms were selected in which abundant material, in all stages of embryonic as well as in adult life, could be easily obtained. These forms are: Petromyzon to represent the available form nearest to Amphioxus; Amblystoma punctatum, among the amphibians; the chick (Gallus domesticus) among the birds; and the pig (Sus scrofa) among the mammals. Other forms, including human material, were studied whenever opportunity offered.

In a word, it may be stated that the hopes held out by the discovery of glycogen in Amphioxus were abundantly fulfilled, for glycogen was found in large amounts in some stage of development in the nervous system of every form studied.

In carrying on the investigation microchemical methods were used, and not the usual analyses of entire animals or organs. On showing microscopical specimens containing glycogen to

chemists it was pointed out by them that where large amounts of glycogen might be present in some elements of the organ or animal, the amount of glycogen relative to the entire bulk might be so small that it would be wholly missed by the usual chemical analyses.   It is also now recognized that chemical analyses by the aid of the microscope have as great validity as those made in the usual way, where relatively large amounts of substance and reagents must be used (Chamot, '15).   Furthermore, the microscopical method is the only one by which the exact anatomical location of the glycogen can be determined.   For the precise steps employed in fixing, imbedding, sectioning, and staining and mounting tissues for glycogen, see the note at the end of this paper.   It may be stated here that to make sure that the mahogany red substance shown in the nerve cells is glycogen, the test was made in every case with saliva, which transforms glycogen to sugar and therefore renders it no longer stainable with iodin; it is believed therefore that the results here given, and which were obtained over and over on many different specimens, can be relied upon.

GLYCOGEN IN THE NERVOUS SYSTEM OF AMPHIOXUS AND ASYMMETRON FROM BERMUDA AND AMPHIOXUS FROM NAPLES

In 1904 while a member of the group of workers at the Bermuda Biological Station, under the direction of Dr. E. L. Mark, advantage was taken of the abundant Amphioxus material there available to investigate the tissues for glycogen.   It was found rather generally distributed but not in striking amounts except in the most unexpected situation, viz., in the large nerve cells of the central nervous system.   This was so opposed to the findings of all previous investigators of glycogen in the nervous system that it was only after repeated verifications on specimens of various sizes that it was accepted.   While any nerve cell apparently might contain glycogen, this substance was most strikingly shown in the large nerve cells associated with pigment.   Whether or not there is any connection, it is a rather striking fact that glycogen in large amount is found in the retinal nerve cells of

adult Petromyzon, in some teleosts Ameiurus, and may be found
in the retinae of higher forms when sufficiently investigated.

Fortunately Bermuda has also in its waters the little Amphi-
oxus discovered by Andrews in the Bahamas (Asymmetron
lucayanum). Asymmetron showed also glycogen in the nerve
cells, agreeing in every particular with Amphioxus. Through
the courtesy of The Wistar Institute, I was enabled to examine
Amphioxus from Naples also, and found glycogen in even greater
abundance if possible in its nerve cells. Probably glycogen is
present in the nerve cells of Amphioxus wherever found.

GLYCOGEN IN THE CENTRAL NERVOUS SYSTEM AND RETINA OF THE LAKE LAMPHREY (PETROMYZON MARINUS UNICOLOR), AND THE BROOK LAMPREY (LAMPETRA WILDERI) OF THE CAYUGA LAKE BASIN

After finding glycogen in the nerve cells of Amphioxus it
seemed probable—at least not improbable—that it might be
found in the nervous system of Ammocoetes (larval Petromyzon
and Lampetra), whose life habits are so similar to Amphioxus,
although living only in fresh water. On returning to Ithaca
from Bermuda, larval lampreys of all sizes were obtained in
nature and put directly into 95 per cent alcohol and some also
in absolute alcohol, exactly as had been done for Amphioxus.
On sectioning and staining this material, glycogen was found as
hoped, and it was in brain cells as well as in those of the myel
(medulla spinalis). Not all nerve cells contain glycogen, but
many of them. In the larval Petromyzon (Ammocoetes) there
was another striking fact brought out in the sections: The
tissue surrounding the central nervous system has the general
appearance of fat; with the glycogen stain almost every cell
showed abundant glycogen in a part of the cell. On using Sudan
III, and osmic acid, as well as the glycogen stain, it appeared
that the cells in the loose tissue enclosing the central nervous
system were most of them filled in part with fat and in part with
glycogen. The cells in the dorsal region of the abdomen around
the mesonephros and gonads were also in many cases partly
surrounded by similar cells filled with fat and with glycogen.

In sections through the entire head of ammocoets the undeveloped eyes were sectioned and much glycogen found in the cones, no rods being present in the petromyzon eye at any stage of development.

The presence of glycogen in the retinal cones of the frog was called attention to long ago by Ehrlich ('83), and recently there has appeared a paper by Brammertz ('15) in which glycogen is asserted to be present in the retinal rods and cones of the frog, the pigeon, and the rabbit.

In the adult Petromyzon and Lampetra, the eye always contains glycogen, but not in the cones.   The glycogen in the functioning eye is in the retinal nerve cells (fig. 4); and very importantly as it appears to me, even in the stages of advanced starvation after the spawning.   Vision seems to be of the highest importance for the lamprey in the shallow streams during its spawning period; and that the vision is good every one will be willing to concede who attempts to catch them.   In addition to the glycogen in the retina proper, the arachnoid layer of the eyeball near the optic nerve is filled with cells containing a large amount of glycogen.   That is much more marked in the lampreys during the vegetative, or growing and maturing period than late in the spawning season.

In passing, attention might be called to a very striking peculiarity of the petromyzon retina.   The optic nerve, instead of passing through all the layers of the retina and finally spreading out on the inside next the vitreous, only extends about half way through the thickness and then spreads out.   As the optic nerve leaves the retina on its way to the brain, the fibers decussate. The lamprey eye certainly deserves more attention than has been accorded to it.

In the course of development of Petromyzon the ova show no glycogen until the eggs are ripened and ready to be shed and, of course, immediately afterward; then the glycogen is abundant and scattered between the yolk granules (fig. 5).   It is in very fine granules and especially abundant near the periphery of the egg.   As the ovum segments, the glycogen is most marked in the mitotic areas of the cells, and as segmentation proceeds it

becomes most condensed at the animal pole and finally in the medullary region which gives rise to the central nervous system. In embryos 5 to 10 mm. long it is marked in the central nervous system. It is at this time present between the granules of food yolk, also in the myotomes, notochord, connective tissue, nephric system, cardiac muscle, liver diverticulum, and epidermis.

In embryos of 10 to 17 mm., in which the food yolk has mostly disappeared, glycogen is present as in the younger embryos, and has appeared in the brain plexus, retina, and auditory epithelium. It is also present in the enteric epithelium.

With larger, well fed specimens, besides the nervous system, the tissues containing glycogen are those of the heart, both auricle and ventricle, branchial epithelium, thyroid duct, branchial cartilages, and their striated muscles, and the muscle of the velum, which has only a striated periphery. The glycogen is in the granular non-striated central part of the velar muscle. In the digestive tract it is found in the gall duct and in the liver; also in the intestinal epithelium, especially the terminal third. In the urinary system it is found in the nephrostomes, and in both pro- and mesonephros, also in the Wolffian duct. It is present in skeletal muscles, the notochord, the primitive skull cartilages, the ear capsule, the fat cells, around the central nervous system and that on the ventral side of the notochord, some of the epidermis, expecially that of the branchial region and the oral hood, the epithelium of the nose and the ear.

AMBLYSTOMA PUNCTATUM

In this salamander, as with most of the Amphibia, the independent life of the young commences very early, and on the alertness in escaping enemies and in obtaining food depends its existence. Going with this early activity is the presence of glycogen in large amount in the unsegmented egg, and in all stages of segmentation. During segmentation glycogen is more abundant in the animal than in the vegetative pole of the ovum. While glycogen is more abundant in the animal pole, as segmen-

tation proceeds and the germ layers are formed it is present in all germ layers but is especially marked in the neural plate.

Glycogen appears in the first proton or anlage of the eye, ear, and nose; in the brain and the myel (medulla spinalis) and in all the organs and tissues of the embryo; that is, in Amblystoma glycogen is universal in its distribution throughout the body in the early embryonic condition, but the liver early takes on the most prominent glycogenic function. It persists for a long time, perhaps throughout life in the cardiac muscle and in the retina (rods and cones).

GALLUS DOMESTICUS

In the chick glycogen appears first in the cardiac muscle, thirty-sixth to the forty-eighth hour of incubation. In strong contrast with Petromyzon and Amblystoma, the appearance of the glycogen in the nervous system is late. In the sixth to the tenth day, it is very abundant in the medulla oblongata and in the sacral and lumbar myel (medulla spinalis).

In the tenth day it appears in cartilage and in the muscles of the trunk and limbs, but it is not so abundant in the somatic muscles of the chick as in those of Petromyzon and mammalian embryos. It is also present, to a limited extent, in the epidermis and the enteric epithelium.

It has already been pointed out by many previous workers that glycogen is not so abundant in the organs and tissues of the chick as in the embryos of many other forms, including mammals. It seems to me that this is true if one deals with the glycogenesis in all of the organs at any one period. With the large amount of stored food in the hen's egg, the chick has the advantage of developing at leisure, so to speak, and whenever the time arrives to bring to definitive form or activity any tissue, the glycogenic builder and energy producer is on hand, but as these perfecting processes do not occur in all the organs and tissues of the chick practically at the same time as with Amblystoma, one finds abundant glycogen at any one period only in a limited region of the body.

GLYCOGEN IN THE NERVOUS SYSTEM OF MAMMALS

Sus scrofa. The pig was selected for determining the presence of glycogen in the nervous system of mammals because of the abundance of material obtainable at all periods of development. Other mammals were examined as opportunity offered, and it was found that whatever occurred in the pig appeared also in other mammals if taken at the favorable developmental state.

Up to the present, glycogen has been found by me in the cells of the dorsal root ganglia of pigs up to a length 15 mm., those of 10 to 12 mm. in length had perhaps the greatest number of nerve cells with the glycogen. At this time the outgrowing nerves seem to be wholly free from glycogen, but commencing with embryos of 30 mm. and as large as 70 mm. and perhaps older ones, the nerves within and beyond the ganglion are so filled with glycogen that they appear a deep brown.

In addition to the nervous element proper, the endymal cells of the relatively free choroid plexus are filled with it in the older embryos, i.e., those of 40 to 70 mm. and perhaps older ones. In addition to the cells on the free plexus, those extending for a considerable distance upon the ventricular wall are well supplied with glycogen.

In the fourth ventricle the endymal cells contain glycogen at a somewhat later stage, viz., in embryos of 50 to 75 mm. in length.

Abundant glycogen was also found in the olfactory as well as the respiratory epithelium of the nose; and its presence is very marked in the epithelium of the cochlear canal opposite the organ of Corti. The eye has not yet been sufficiently studied, but the appearance of glycogen at some period is predicted. Glycogen was found in every organ and tissue in the body of pig embryos at some period of development. Naturally the heart contained much of it. For example, in the smaller pigs studied, i.e., those of 8 to 16 mm., the cardiac glycogen was so abundant that it made the heart sections almost opaque. In embryos of 70 mm. the amount was relatively less. The liver contained no

glycogen in any of the embryo pigs studied, thus agreeing with the statements of Bernard that glycogen is relatively late in appearing in the liver.

In the alimentary canal the glycogen passes as a kind of wave along the tube, commencing at the mouth and passing in order to the esophagus, the stomach, and, as the villi commence to appear, extending along down the small to the large intestine.

The investigation of human embryos for glycogen is carried on with more uncertainty than is that for other forms owing to the difficulty of obtaining material in the different stages which can be fixed in the alcohol before the glycogen becomes dissolved. However, owing to the courtesy of Dr. Mall, and several of my old students, some fairly normal human embryos fixed in alcohol before all of the glycogen was dissolved, have been studied, and I have found the glycogen distributed among the organs and tissues as described for mammals generally.

In the nervous system, the only unmistakable situation in which it has been found up to the present is in the choroid plexus of a 19 cm. human fetus and in the choroid plexus and the cells of the raphé of the medulla oblongata of a 35 mm. human embryo preserved in strong alcohol. The endymal cells showed the same abundance of glycogen that has been found in the embryo of the cat and pig. A figure of this human plexus with the glycogenated endymal cells is given in the accompanying plate (fig. 10). It is confidently expected that when the proper material can be obtained glycogen will be found in the human nervous system and organs of sense, as with other mammals.

CONCLUSIONS

From the data given above it is believed that the following conclusions may be fairly drawn:

1. The production and use of glycogen is one of the properties of nervous as of all other forms of protoplasm.

2. Glycogen is an essential accompanier of nervous as of all other tissues in their histogenesis, especially in the transition to their definitive and functional stage.

3. Glycogen is, then, a builder as well as an energy producer for nervous as for all other forms of tissue.

4. Its appearance in developing tissue in all forms of vertebrates depends in part, at least, upon the relative time in which the tissues must function. For example in Amblystoma that must have full functional activity very early in its life, the perfecting glycogen appears correspondingly early, while with the chick it is late in appearing.

5. After reaching their definitive form the elements of the nervous system in the lowest vertebrates, Amphioxus and larval lampreys (Ammocoetes), continue their glycogenic function in the central nervous system. In the adult lampreys (Petromyzon and Lampetra), this function persists throughout life in the nerve cells of the retina. With the higher vertebrates, glycogen in demonstrable amount is not found in the nervous system after the embryonic period, the liver and muscles then assuming the main glycogenic function.

That is, specialization of this function keeps pace with differentiation of structure consequent upon advance in the zoölogical scale.

METHOD OF DEMONSTRATING GLYCOGEN

The fundamental thing is that no liquid is to be used in any of the steps that will dissolve the glycogen. The most certain medium for fixing is alcohol. Absolute alcohol is mostly recommended; but, as glycogen is wholly insoluble in alcohol of 67 per cent and, of course, in all stronger grades, one has considerable range.

As alcohol diffuses through the tissues slowly, only small animals and small embryos should be fixed entire. For the organs and tissues of larger forms small pieces or widely opened and dissected organs in which the alcohol comes quickly in contact with all the parts containing glycogen should be used. Plenty of alcohol should be used—fifty times the bulk of the tissue—and it is well to change it two or three times. In general it is safer to use alcohol of 95 per cent, then it is not liable to be diluted by the lymph sufficiently to make the glycogen soluble.

As alcohol distorts the tissues, it is well to carry along parallel specimens prepared by the usual fixers. Mercuric chlorid, or mercuric chlorid and dichromate mixtures are good. Picric alcohol is also good and it has the advantage of fixing the glycogen as well as the other tissue elements (it is composed of 67 per cent alcohol, 500 cc.; picric acid, 1

gram). The tissue is fixed in this twelve to twenty-four hours and then transferred to 67 per cent alcohol the same time; and then for a day or more in 82 per cent alcohol before the final imbedding. Plenty of fixer and alcohol should be used.

Other fixers have been recommended for glycogen, and many different ones preserve a part of the glycogen, but as the purpose of any investigation on glycogen is to find all the elements in which it is present either in large or in small amounts, a fixer should be used which experience has shown to be the most precise and certain, and that fixer is alcohol.

For large embryos, small animals, limbs, etc. containing bone it was found entirely practicable to decalcify the bone without in any way disturbing the glycogen. The embryo, animal or part is fixed with alcohol as usual for glycogen, then it is placed in the nitric acid decalcifier composed of 67 per cent alcohol to which has been added 3 per cent nitric acid. After the decalcification is complete, the embryo remains a day or two in 67 per cent alcohol, changed two or three times. It is then transferred to 82 per cent alcohol for a day or more before dehydrating and imbedding.

Imbedding and sectioning. Either the collodion or the paraffin method can be used. The paraffin method or the combined collodion and paraffin method has proved most satisfactory in my work. Some of the sections should be moderately thick, 10 to 15 μ. Sections less than 5 μ are not serviceable for glycogen investigations.

Staining glycogen. The only fully reliable and satisfactory stain for glycogen is iodin. As some glycogen is very soluble, a glycogen stain containing alcohol was found most generally useful (95 per cent alcohol, 150 cc.; water, 150 cc.; iodin crystals, 1.5 grams or 15 cc. of a 10 per cent alcoholic solution of iodin; iodid of potassium, 3 grams; sodium chlorid, 1.5 grams). For the aqueous stain, water is used instead of the alcohol mixture.

For staining, spread the paraffin sections with the iodin stain instead of water. The glycogen in the sections will stain a mahogany red and the stain will remain in the spread sections for years (10 to 15). If care is taken not to melt the paraffin when spreading the sections, they can be restained at any time by immersing the slide in the stain or placing some of the stain on the sections.

Permanent preparations. The permanence of the iodin stain in the spread paraffin sections gave the clue. For low powers the sections in paraffin show very well without further preparation, but for high powers the crystals of paraffin interfere. Various paraffin media were tried, and finally ordinary yellow vaseline was settled upon as best. For mounting, the sections are restained by immersing the slide in the iodin stain for two to three minutes or longer; they are then dried half an hour or more in the air or in a drying oven, then immersed in xylene to dissolve off the paraffin. Some melted yellow vaseline is then put on the sections and a cover-glass added exactly

as in balsam mounting. As the vaseline does not hold the cover very firmly, it is best to seal the cover with shellac.

Specimens so stained may be restained at any time by reversing the process and then remounting. The stain remains for two to ten years.

A second method was to mount in Canada balsam without a cover-glass, as with the Golgi preparations. For this dried balsam is powdered and to 25 grams of dry balsam, 50 cc. of xylene is added. The sections are deparaffined, and the balsam put over them and allowed to dry in the air. More than one coat of balsam may be needed.

The glycogen stain is not so persistent in the balsam, but for high power work the finest details are more satisfactory. If a homogeneous immersion objective is to be used the original immersion liquid, viz., Canada balsam of moderate thickness is better than cedar oil. It need not be removed. The other stains recommended for glycogen are not so precise as iodin, and are liable, if not checked by iodin, to lead the investigator astray.

The most exact test for glycogen is saliva. If a section is deparaffined, washed off with alcohol and water, and then saliva put upon it for half an hour, if the substance is glycogen it will be changed to sugar by the enzyme of the saliva. If now the section is restained with iodin no mahogany red glycogen will appear. This test was applied to all the work given in the accompanying paper to make sure that the reddish brown substance in the cells was glycogen and not something else.

BIBLIOGRAPHY

The literature of glycogen is very extensive, and may be found in the Index Catalogue of the Library of the Surgeon General's Office, Ser. I, II, and in special papers such as Fichera's with its 311 references and in Pflüger's book on Glycogen. Only the works bearing directly upon the subject of this discussion are here given.

BARFURTH, D. 1885 Vergleichend-histochemische Untersuchungen über das Glycogen. Arch. f. mikr. Anat., Bd. 25, pp. 261–404. Nervous system of vertebrates, pp. 297, 299. Invertebrates, p. 298. While denying glycogen to vertebrate nervous tissue at any period, p. 299, he reported its presence in small amounts in the nervous system of snails.

BERNARD, CLAUDE 1859 De la matière glycogène considerée comme condition de développement de certain tissus chez le foetus avant l'apparition de la fonction glycogénique du foie. Jour. de la Physiol. t. 2, Pp. 326–337.

1878–1879 Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux. Two volumes.

Bernard wrote many papers upon sugar in the blood and upon glycogen, which he discovered and isolated in 1857. The paper cited above and the volumes on the phenomena of life give his views very

completely. It may be said in passing that physiologists have not gone far beyond Bernard. It is however, more widely distributed than he thought, and its locations have been more completely mapped out since his day.

BRAMMERTZ, WM. 1915 Ueber das normale Vorkommen von Glykogen in der Retina. Arch. f. mikr. Anat., Bd. 86, pp. 1–7. He reports the presence of glycogen in the rods and cones of frog, pigeon, rabbit and between them in the pike, and in the eye of the house fly.

CREIGHTON, CHARLES 1896–1899 Microscopic researches on the formative property of glycogen. Part I with five colored plates, 152 pages. Part II ('99), Glycogen of snails and slugs. Nine plates, 127 pages. In Part I he figures and discusses glycogen in the choroid plexus of the cat embryo, pl. i, figure 3; and a combination of fat and glycogen in the developing mammary gland, pl. iv, figure 20. All students of glycogen would do well to consult this work.

CHAMOT, E. M. 1915 Elementary chemical microscopy.

DUGGAR, B. M. 1911 Plant physiology. This work gives a good account of starch and its transformations in plants.

FICHERA, G. 1904 Ueber die Verbreitung des Glycogens in Verschiedenen Arten. Ziegler's Beiträge zur path. Anat., Bd. 36, pp. 273–339. Fichera gives references to 311 papers.

GAGE, S. H. 1905–1906 Glycogen in the nervous system. Reports and demonstrations at the Association of American Anatomists. Am. Jour. Anat., 1905, pp. xii–xiii; 1906, pp. xiii–xv.

LUSK, GRAHAM 1906 The elements of the science of nutrition. Discusses carbohydrates, including glycogen, in nutrition.

MENDEL, L. B. AND LEAVENWORTH, C. S. 1907, The American Journal of Physiology, vol. 20, pp. 117–126. Chemical studies on growth; III, the occurrence of glycogen in the pig. They used the brain substance in rather large amounts and got only negative results. Their final conclusion is that embryos do not have a large amount of glycogen in their tissues. The author showed Dr. Mendel the microscopic sections of embryo pigs and he could not help thinking that a great deal of glycogen was present. In case of the brain it is entirely possible that the amount of glycogen in the choroid plexus might not be sufficient to give results in ordinary analyses, although they are most striking and convincing in microscopic preparations.

PFLÜGER, E. F. W. 1905 Das Glykogen und seine Beziehungen zur Zuckerkrankheit. Zweite Auflage. Very many references.

SMITH, LUCY WRIGHT 1912 Glycogen in Insects, especially in the nervous system and eyes. Science, vol. 35, March 1. Much glycogen is found in both compound and simple eyes and in the nerve cells of the ganglia from all parts of the body, but not in the nerve fibers.

1 Glycogenated nerve cells of Amphioxus.

2 Nerve cells of Ammocoetes from the brain opposite the eye and ear.

3 Nerve cells in the myel (medulla spinalis) of Ammocoetes with glycogen; also glycogen and fat in the same cells.

4 Retina of an adult Petromyzon showing glycogen in the retinal nerve cells, and the decussation of the optic fibers.
(a) Edge view of glycogenated retinal nerve cells.
(b) Face view of retinal nerve cells.

5 Ova of Petromyzon and Amblystoma (bc) showing the great amount of glycogen in the cells of the animal pole. In the developing nervous system there is much glycogen.

6 Lumbar enlargement of a ten day chick's medulla spinalis showing a prismatic mass of glycogenated cells in the raphé. A similar glycogenated area is present in the medulla oblongata.

7 Spinal ganglion of a 12 mm. pig embryo with glycogenated cells. (a) Some of the cells greatly enlarged.

8 Glycogenated nerve trunks of the brachial plexus of a 30 mm. pig embryo.

9 Glycogenated endymal cells of the brain plexuses in a cat embryo. (a) Endymal cells greatly enlarged.

10 Choroid plexus of a 19 cm. human embryo. (ab) Section through the cerebrum and medulla of a 35 mm. human embryo to show the glycogenated choroid plexus and the cells of the raphé in the medulla.

SIMON H. GAGE

THE MOTOR NUCLEI OF THE CEREBRAL NERVES IN PHYLOGENY: A STUDY OF THE PHENOMENA OF NEUROBIOTAXIS

PART I. CYCLOSTOMI AND PISCES

DAVIDSON BLACK

From the Central Institute for Brain Research, Amsterdam, Holland, and the Anatomical Department, Medical School, Western Reserve University, Cleveland, Ohio

FORTY-TWO FIGURES

CONTENTS

INTRODUCTION

In 1907 Kappers first directed attention to the significance of the phylogenetic displacements of the motor nuclei of the medulla. In 1908 (60, p. 521) he enunciated more definitely his doctrine of Neurobiotaxis and set forth the following conclusions deduced from the study of nuclear migrations:

1. Wenn in dem Nervensysteme am verschiedenen Stellen Reizladungen auftreten, so erfolgt das Auswachsen der Hauptdendriten,

467

namentlich auch die Verlagerung des ganzen Leibes der Ganglienzellen, in der Richtung der maximalen Reizladung.

2. Nur zwischen gleichzeitig oder direkt sukzessiv gereizten Stellen findet diese Dendriten- oder Zellenannäherung statt.

3. Das Auswachsen der Achsenzylinder der sogenannten Zentral-motorischen Systeme wird nicht primär bedingt durch die motile Funktion gewisser Zellen, sondern ebenfalls durch synchron oder sukzessiv gereizte Gebiete (Schaltzellen v. Monakows).

Since that time much work has been done by this author and his associates in extending and elaborating the field of these investigations, the importance of which, in view of their wide application to the problems of morphology, ontology and physiology of the nervous system, becomes increasingly evident.[1]

It was my privilege to begin the present work under the direction of Dr. Kappers in the summer of 1914 with the object of adding to the data bearing upon the phenomena of neuro-biotaxis, by the study of new material recently acquired by the Central Dutch Institute for Brain Research. In accordance with this object the plan of treatment here adopted corresponds somewhat to that obtaining in Kappers' communication of 1912 (66) and the reconstruction charts of the motor nuclei in both cases are of the same dimensions. With regard to plotting these charts, it seems hardly necessary to point out that all charts can be made to correspond approximately in size for the sake of direct comparison because a difference in magnification does not in any way alter relative proportion. A description of this method of reconstruction and a discussion of its advantages and limitations will be found in Kappers' earlier papers. A brief statement concerning the method is also set forth in the present article on page 474.

Except in the case of Solea vulgaris, all the observations, drawings and reconstructions were carried out in the laboratories of the Central Dutch Institute in Amsterdam. My work being unavoidably interrupted in August, 1914, Dr. Kappers very kindly gave me the slides of the Solea series so that I might subsequently complete my observations on this form.

[1] Reference may be had to the chief communications on the subject of Neuro-biotaxis in the appended bibliography.

It is a pleasure for me to acknowledge my indebtedness to Dr. Kappers, as well for his help and the stimulating interest he took in my work as for the generous way in which he placed the resources of his laboratory at my disposal.

With regard to my material, the present work is based upon the special study of the motor nuclei in the following representative forms: Bdellostoma dombeyi, Selache maxima (Cetorhinus maximus), Polyodon spathula, Ameiurus nebulosus, Solea vulgaris, Rana catesbeana (mugiens), Damonia subtrijuga, Cacatua roseicapilla, Hypsiprimnus murinus and Pan satyrus (Troglodytes niger). In addition, Hexanchus, Heptanchus, Ciconia alba and Vesperugo noctula were re-studied, though the motor nuclei in these forms had already been charted and recorded elsewhere.[2]

The specimens of Polyodon spathula were obtained through the courtesy of Prof. R. J. Terry of Washington University, St. Louis, and I am also much indebted to Prof. Howard Ayers who very generously furnished me with specimens of Bdellostoma dombeyi from his personal collection. Several of these brains had been fixed by him by the Cajal method, others fixed by different methods were subsequently stained in various ways.

The series for the most part were cut transversely at 25 microns. With the exception of Bdellostoma, alternate sections in each series were arranged on celloidin films and stained by Pal-Weigert-carmine and van Giesson methods (Kappers, 64).

All the technical work in connection with cutting, staining and mounting this material, was done in the laboratories of the Central Dutch Institute for Brain Research and I wish to ex-

---

[2] The name 'Pan satyrus' is used here for this species of chimpanzee in conformity with the recent work of the late D. G. Elliott (21). The claims of priority also demand the change in nomenclature from 'R. mugiens' to 'R. catesbeana' for the bull frog (vide Gadow, 26). In the case of the sharks Notidanus cinereus and N. griseus, the old terms Heptanchus and Hexanchus respectively have been retained for convenience in description, while in the case of the basking shark the name 'Selache maxima' rather than 'Cetorhinus maximus' has been used, though both seem equally in vogue (vide Jordan, 54, and Bridge, 12).

press here my sincere appreciation and thanks to Miss de Lange for the skilled and careful manner in which she carried out this important part of the work.

CYCLOSTOMI

Motor nuclei in Bdellostoma dombeyi

This form is the common hagfish of the American Pacific coast which, according to Worthington, is identical with the Californian variety not infrequently described as B. stouti (100). Several brains of this form prepared by different methods and cut in both transverse and sagittal series were studied. The reconstruction chart (fig. 7) was prepared from one series cut transversely and stained by the method of Cajal.

Spino-occipital nuclei and roots (Nu. et rad. mot. Nn. spin. occ.) At the junction of the cord and medulla in Bdellostoma certain very definitely specialized nerves may be recognized, each possessing one sensory and two motor roots. The essential difference between these specialized nerves and the cervical motor roots lies in the total absence in the former of peripheral branches supplying dorsal trunk musculature (Worthington, 99). Furbringer's term 'spino-occipital' (24) has been adopted by Worthington to describe these nerves and this name has been retained in the present description.[3]

Both the motor roots of the first spino-occipital nerve have their superficial origin slightly caudad of the level of entrance of the sensory root, while the reverse is the case in the second spino-occipital nerve. In the latter nerve, four motor rootlets uniting to form two main stems were described by Worthington (99) but I was unable to confirm this observation.

---

[3] However, it is evident that the nerves in question are derived from segments more rostrally placed than those from which the first two spinooccipital roots arise in selachians (Neal, 84, Furbringer, 25, et. al). Thus, both here and in the subsequent description of this region in selachians and ganoids, the term 'spino-occipital' is used in its broadest collective sense to designate certain precervical motor elements whose number and segmental relationships are subject to considerable variation in these different groups.

The motor roots of the spino-occipital nerves course obliquely cephalad and mesiad to reach their nuclei of origin in the terminal rostral portion of anterior horn of the cord (fig. 1). There is no indication whatever of any separation between these nuclei and those of the succeeding ventral spinal nerves.
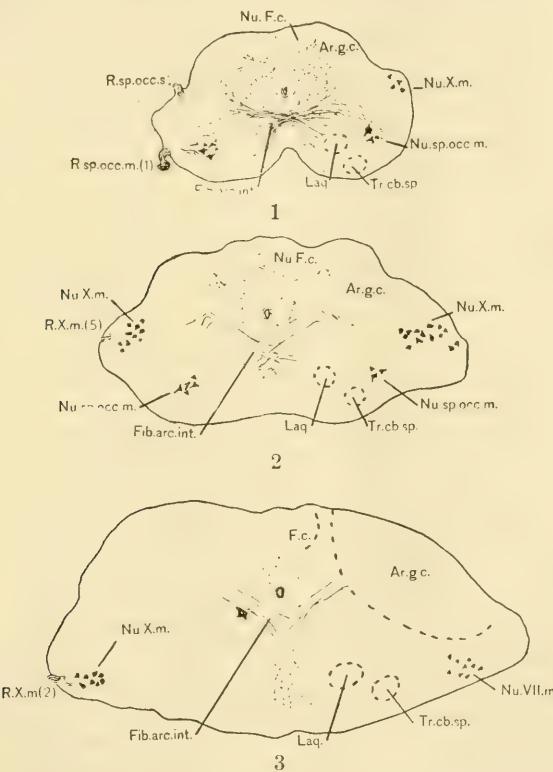
Figs. 1 to 3 Bdellostoma dombeyi. Outlines of transverse sections to illustrate the topography of the medulla. Sensory areas and fiber tracts indicated diagrammatically. Figures 1 to 6 drawn to same scale. Abbreviations: $Ar.g.c.$, general cutaneous area; $F.c.$, fasciculus communis; $Fib.arc.int.$, fibrae arcuatae internae; $Laq.$, laqueus; s. tractus tecto-bulbaris et spinalis; $Nu.f.c.$, nucleus and region of fasciculus communis; $Nu.sp.occ.m.$, rostral portion of anterior horn (spino-occipital motor nucleus); $Nu.VII.m.$, caudal end of nucleus motorius N. facialis; $Nu.X.m.$, nucleus motorius N. vagi; $R.sp.occ.m.(1)$., first motor rootlet of first spino-occipital nerve; $R.sp.occ.s.$, part of sensory root of first spino-occipital nerve; $R.X.m.(2)$., second motor rootlet of vagus; $R.X.m.(5)$., fifth motor root of vagus: $Tr.cb.sp.$, tractus cerebello-spinalis.

Worthington (l.c.) described a continuation upward of the anterior horn nucleus almost to the rostral end of the medulla and noted the presence of a number of large cells of 'Mauthner' in the upper part of the nucleus. This is probably homologous with the nucleus that Holm observed in Myxine and to which he gave the name "ganglion centrale nucleus posterior" (45). Worthington states, moreover, that the nucleus "gives fibers to the vagus," while Holm makes it evident that he did not consider this cell group to be a nucleus of origin for peripheral motor fibers.

I have examined this cell column rostral to the origin of the motor spino-occipital roots in Bdellostoma and have indicated some of its largest Müller cells in figures 1 to 6. I could not discover any evidence of the direct connection of these cells with the roots of the vagus series. They do, however, send processes into the neighborhood of the viscero-motor column and ventro-lateral area of the bulb.

Thus, in the present connection I cannot consider this cell group to be a part of the somatic motor column proper; it is, rather, of the nature of a scattered reticular nucleus of specialized coordinative function,[4] such as is commonly found near the raphé in the caudal part of the medulla in all vertebrates (van Hoevell, 44).

However, this reticular nucleus in Bdellostoma in all probability is the specialized representative of the somatic column which has lost, or is in process of losing, its peripheral motor components but has retained its primitive dorso-mesial position and its intercalary coordination elements. This view would be supported by the important observations of Bartelmez on the nucleus motorius tegmenti in Ameiurus (9) which is apparently represented in Bdellostoma by the reticular nucleus under discussion. According to this author the nucleus motorius tegmenti, together with the nucleus abducentis, constitutes the somatic motor column of the bulb.

---

[4] The terms 'coordinative' and 'correlative' are used in the present paper in the sense defined by Herrick (42, p. 35).

It is quite possible that further study of a large number of specimens may confirm Worthington's observations and show that the spino-occipital nucleus overlaps the vagus area in some specimens to a greater extent than in the one charted in figure 7. Individual variation, especially in this part of the brain in Bdellostoma, is rather to be expected, so that Johnson's observation that "the segment of the glossopharyngeus also has a somatic motor nerve in Bdellostoma" (52, p. 194) should be modified to a certain extent.

Motor vagal nuclei and roots (*Nu. et rad. mot. N. X*). The remaining motor nuclei in this form are visceral in character and may conveniently be described as forming two columns of cells, one on each side of the bulb in the ventro-lateral area. Each column is further definitely subdivided into a rostral and a caudal portion (vide fig. 7).

The caudal viscero-motor column corresponds to the caudal atero-ventral column described by Holm in Myxine (l.c.). It is the motor nucleus of the vagus and consists of a continuous column of medium sized cells from which five (?) vagus rootlets arise. It is probable that the first two very small rootlets should be considered together as the first vagus root, though the first small rootlet of this series in all probability represents the motor glossopharyngeus of other authors (q. v.). The presence of a true glossopharyngeal nerve in Bdellostoma, however, is highly improbable (vide infra.).

Rostrally the vagus nucleus begins a few sections above the level of its first motor rootlet, while caudally it extends for some distance below the level of the upper prolongation of the anterior horn.

Motor trigemino-facial nuclei and roots (*Nu. et rad. mot. Nn. V–VII*). The rostral viscero-motor column (frontal latero-ventral column of Holm) contains the motor nuclei of the trigeminus and facialis (fig. 7 C).

The motor root of the trigeminus consists of two parts, of which the rostral is the larger. These two portions are only evident on the periphery of the bulb and at once unite to form a single trunk. The motor nucleus of this nerve is also divisible

into two parts: a large celled nucleus (nucleus magnocellularis) extending approximately over the length of the superficial origin of the motor trigeminal roots, and a smaller celled nucleus (nucleus parvicellularis) which is incompletely separated from the former and becomes directly continuous caudally with the motor facial nucleus (figs. 4, 5 and 6).

I have been unable to demonstrate in Bdellostoma such a complete correspondence between the two motor roots of the trigeminal nerve and the two cell groups of its nucleus of origin, as was done in the case of Myxine by Röthig and Kappers (88). It is presumable, however, that the first root takes its chief origin from the nucleus magnocellularis, though some of the fibers of the second root appear to originate there also.

The motor facial nucleus may be traced down to within a short distance of the rostral end of the vagus column. It is difficult to set a definite limit to this nucleus caudally, as the cells in this part gradually become scattered. No root fibers from any source, however, can be traced into this diffuse caudal portion, which occupies a position somewhat more mediad than the caudal visceral motor nucleus.

The abducens, trochlear and oculomotor nerves are absent in Bdellostoma, in which there is no functional eye and consequently no intrinsic nor extrinsic musculature to require innervation.

Method of reconstruction. The relations described above are graphically represented in figure 7 C. The nuclei and motor roots in question are plotted as if projected upon the midsagittal plane. In view of earlier discussions of this method of reconstruction, it is only necessary to mention here briefly the following points:

1. Only the nuclei of one side of the brain stem (right or left) are plotted in these charts.

2. The longitudinal extent and relations of the nuclei and emergent roots are accurately plotted.

3. The dorso-ventral extent and relations of the nuclei are indicated only approximately. For this reason the charts have been supplemented by projectoscope drawings of transverse

sections of the brain stem taken at various representative levels
in the series, where these relations can be accurately recorded.

4. For the sake of convenience the general outlines and
shapes of the nuclei are depicted quite diagrammatically.   In
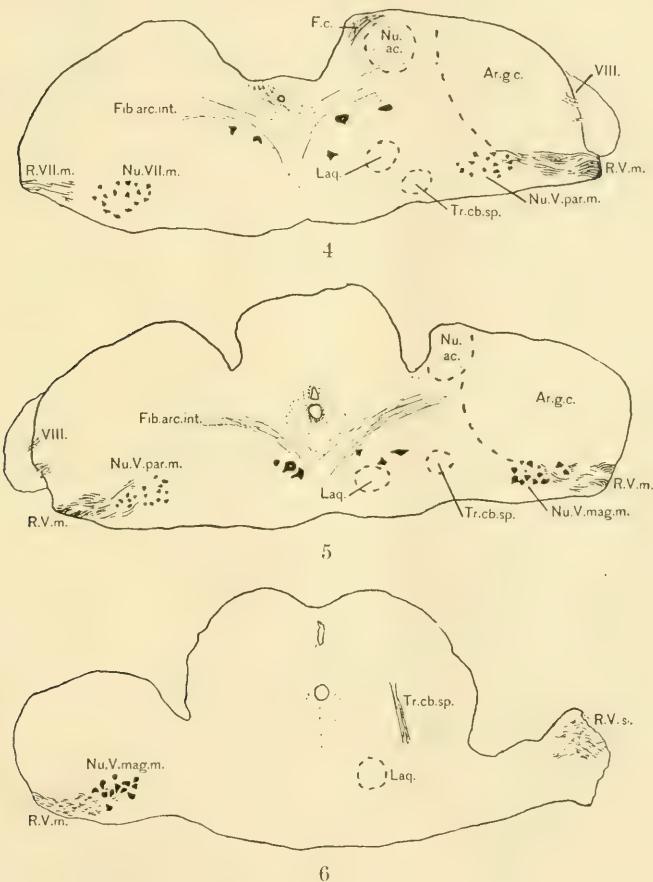
Figs. 4 to 6   Bdellostoma dombeyi.   Outlines of transverse sections to illus-
trate the topography of the medulla.   Sensory areas and fiber tracts indicated
diagrammatically.   Abbreviations: *Nu.ac.*,   nucleus   acusticus;   *Nu.V.mag.*,
nucleus motorius N. trigemini (magnocellularis); *Nu.V.paf.*, nucleus motorius
N. trigemini (parvicellularis); *R.V.m.*, radix motorius N. trigemini; *R.VII.m.*,
radix motorious N. facialis; *VIII.*, acoustic fibers.   Other abbreviations as in
figures 1 to 3.

this connection reference should be made to the exact nuclear reconstructions made by Berkelbach Van der Sprenkel and to the discussion by this author of the method he employed (92).

In not a few instances, nuclear masses other than those of the motor nerves have been plotted on the charts. This has been done, for example, in the case of the superior olive and inferior olive (nucleus paramedianus) in such forms as they occur in a sufficiently well differentiated condition to allow a sharp demarcation. Similarly the site of the tip of the calamus scriptorius is indicated in all forms with the exception of myxinoids, where a true calamus is absent. The addition of these landmarks has been made to facilitate the comparison of different forms one with another by increasing the number of points for comparison.

Fig. 7 Reconstruction charts of motor roots and nuclei: (A), Petromyzon fluviatilis (after Kappers, 66). (B), Myxine glutinosa (after Röthig and Kappers, 88). (C), Bdellostoma dombeyi. Explanation of signs and abbreviations used in all charts as follows: *VI.*, abducens root; *IX.*, motor glossopharyngeal root; *X.*, motor vagus rootlets; *Sp.occ.*, motor spino-occipital rootlets. The arrow indicates the site of the calamus.

Oculomotor root and nucleus

Trochlear root and nucleus

Trigeminus motor root and nucleus

Abducens nucleus

Facial motor root and nucleus

Glossopharyngeus motor nucleus

Vagus motor nucleus

Spino-occipital motor roots and nucleus

Nu.olivaris superior

Nu.paramedianus
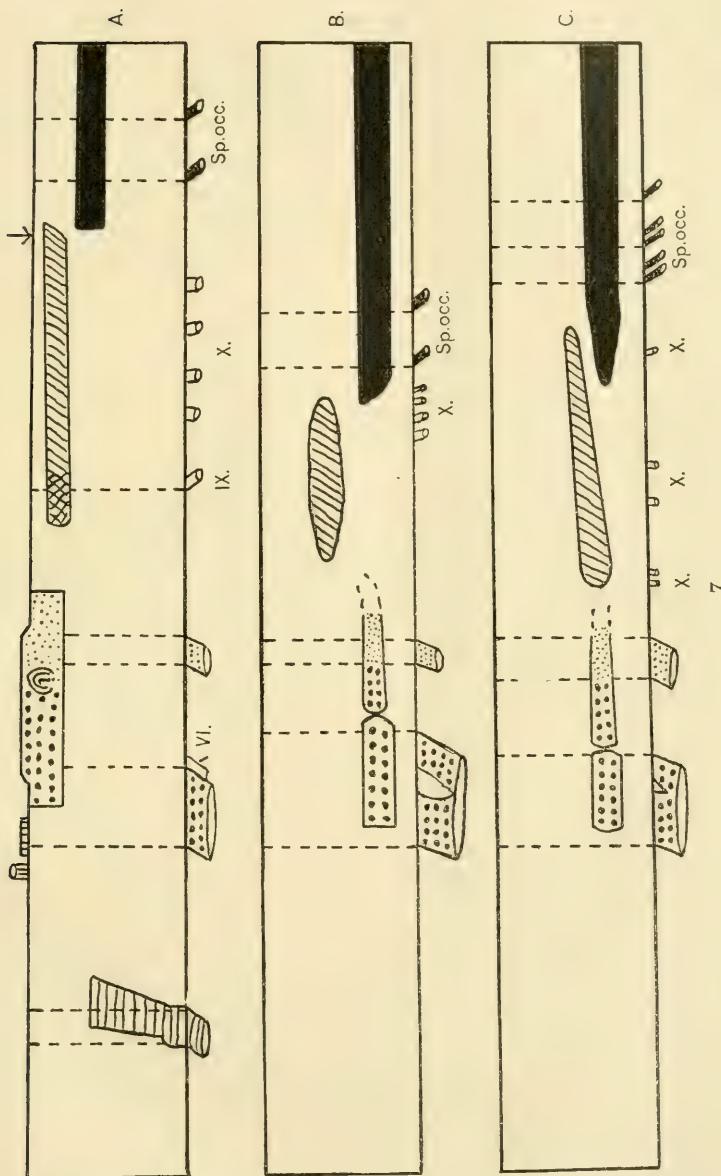
Discussion

On comparing the reconstruction of the motor nuclei in Bdellostoma dombeyi with that of the nuclei in Petromyzon fluviatilis (fig. 7), two striking differences are at once apparent: firstly, the ventral position of all motor nuclei in Bdellostoma, and secondly, the complete absence in the latter form of the oculomotor, trochlear, and abducens nerves. In these two respects Bdellostoma closely agrees with Myxine glutinosa (vide fig. 7, B).

In all these forms there is a significant division of the visceral motor nuclei into two parts: (a), a caudal portion, consisting of the vagus and glossopharyngeus nuclei in Petromyzon, and of the vagus nucleus alone in Bdellostoma and Myxine; and (b) a frontal portion, consisting of the V–VII nucleus.

It is important to note that the position of the last named nucleus with reference to the level of exit of the V and VII motor roots, is almost exactly similar in each of the three forms.

It is strongly probable that in phylogeny all motor nuclei tend to be situated originally on the segmental level of their emergent roots and close to, or within the central gray. Kappers has already drawn special attention to this point. Thus, in Petromyzon the position of the chief bulk of the motor VII nucleus in the central gray, approximately on the exit level of its motor root, is indicative of a primitive condition. The fusion of the V–VII motor nuclei (or it may be, their non-separation) Kappers also considers somewhat primitive for various reasons, though the possibility of such a condition is to a certain extent no doubt dependent upon the absence of a large gustatory VII–IX–X center in these forms (see especially 61 and 64).

The ventro-lateral displacement of the motor V–VII nucleus in Myxine has occurred under the dominating influence of the chief reflex paths (especially the general cutaneous system) situated in the lateral and ventral area of the medulla (Röthig, 87, Röthig and Kappers, 88).

It will be of interest then to inquire into the arrangement of the chief reflex connections of the motor V and VII nuclei in

Bdellostoma and consider in how far the position of these nuclei presents evidence of the operation of neurobiotactic forces.

Worthington has clearly shown that in Bdellostoma the two chief sources of information concerning its environment are respectively olfactory and tactile (100).

The whole brain rostral to, and including, the so-called mid-brain is dominated, almost exclusively, by the olfactory apparatus. On the other hand, the largest and most important tract of the hindbrain, one which dominates all the anatomical arrangement of the medulla and extends from the funicular nuclei to the level of the large trigeminal sensory root, is the general cutaneous system (99). Worthington remarks further that "this tract," (ascending sensory trigeminal) "judging from Johnston (51), is relatively much larger in Bdellostoma" than it is in Petromyzon (l. c., p. 160).

The fasciculus communis system in Bdellostoma, though definitely developed, is small (Ayers and Worthington, 5), while the same may be said of the acustico-lateral system (Ayers and Worthington, 4). Both these, like the general cutaneous system, give rise to efferent fibers passing either by way of the fibrae arcuatae internae to the region of the heterolateral viscero-motor nuclei, or directly ventral to the homolateral motor column.

Moreover these authors have shown that a free and extensive system of intercommunications between the general cutaneous, communis, and acustico-lateral areas by means of correlation neurones, is characteristically developed. In addition to these connections it would appear from the description and figures of these authors (4, p. 7 and 8, figs. 38, 40 and 43) that projection neurones also are related by their dendrites to each of the three areas mentioned. It follows from this that the secondary correlation tracts in the brain of Bdellostoma can not possess a very high degree of specificity of function.

Thus, a condition exists in this form which is apparently not far removed from that obtaining in the medulla of the half-grown amphibian larva, where each correlation tract "may be actuated physiologically at the same time by two or more diverse physiological systems of the periphery" (Herrick and Coghill, 43).

In this connection it is of interest to recall Worthington's observations (q. v.) that Bdellostoma possesses but two characteristic reactions toward injury or discomfort, viz., wriggling (swimming) and casting off slime (c. f. common type of total response of Amblystoma larva, Herrick and Coghill, l. c.).

The fasciculus longitudinalis posterior is but slightly developed, as may be expected, and practically all the chief secondary correlation tracts course in the formatio reticularis of the ventro-lateral area of the bulb.

The tractus tecto-bulbaris et spinalis (laqueus of Holm) is without doubt primarily olfactory efferent in character since the so-called midbrain is comparable in but few, if any, respects with the structure bearing this name in higher forms. The chief afferent connections of the midbrain are from the habenular nuclei and the base of the forebrain, both of which are olfactory nuclei. The laqueus descends through the brain stem in the ventro-lateral area and passes into the cord. According to Holm's descriptions and figures, this tract in Myxine lies about midway between the raphé and the viscero-motor column.

The so-called cerebellum has its chief afferent connections by way of the tractus olfacto-cerebellaris from the floor of the forebrain and by crossed and direct fibers from the acoustic nuclei of the medulla. The greatest efferent path from this region is the tractus cerebello-spinalis which passes through the upper part of the general cutaneous nucleus and then courses in the lateral part of the formatio reticularis beneath this nucleus.

A tract from the interpeduncular region, also olfactory in character, passes down an indefinite distance in the ventral formatio reticularis of the medulla.

The arrangement of these tracts, etc., is illustrated diagrammatically in figures 1 to 6.

The ventro-lateral formatio reticularis thus contains the chief olfactory projection paths. Dorsal to this region, the great general cutaneous area and the subsidiary communis and the acoustico-lateral centers are located. Between the overlying general cutaneous area and the ventro-laterally placed olfactory paths, the whole viscero-motor column in Bdellostoma is situated.

The viscero-motor column is situated in the midst of the undifferentiated formatio reticularis, through which must pass the majority of intrasegmental impulses, acting reflexly upon it from the overlying general cutaneous and associated areas. The presence of the long olfactory paths in the ventro-lateral area is more probably the result rather than the cause of the position of the viscero-motor column.

The ventro-lateral displacement of the V–VII, as well as the X nucleus, from the primitive dorsal position has thus been in the direction of the greatest number of incoming impulses in accordance with the first concept of neurobiotaxis (vide supra).

With regard to the close association of the motor nuclei of the trigeminus and facialis to form the rostral visceral motor column it is to be noted that these nuclei innervate musculature derived from the maxillary, mandibular and hyoid arches which is primarily concerned in the ingestion of food and not in respiration. During the process of feeding the musculature in question has no respiratory function whatever and respiration takes place at such times by ebb and flow of water through the gill clefts.

The chief feeding reflexes in Bdellostoma are primarily initiated by tactile stimuli from the tentacles which are innervated exclusively by the trigeminal nerve (3, 100).

The fusion (or perhaps the non-separation) of these two effector nuclei (V–VII) whose coordinate action is initiated by similar and almost simultaneous afferent impulses, may thus illustrate the second concept of neurobiotaxis (vide supra).

A comparison of the total length of the caudal viscero-motor column in Petromyzon with that in Bdellostoma brings out the fact that the column in question is shorter in Bdellostoma than in Petromyzon by just the length of the IX nucleus. In other words, the vagus nucleus proper in Petromyzon is almost exactly the same length as the entire caudal viscero-motor column in Bdellostoma (fig. 8).

The gap between the rostral and caudal viscero-motor column is smaller in Bdellostoma than in either Petromyzon or Myxine; while the caudal viscero-motor column overlaps the anterior horn (spino-occipital nucleus) to a considerable extent in

Bdellostoma, though this is not the case in either Petromyzon
or Myxine.

The number of gill sacs in petromyzonts is seven, while in
Myxinidae the gill sacs seldom exceed six pairs, though in rare
cases there may be seven pairs present (Bridge, 12, p. 422, p.
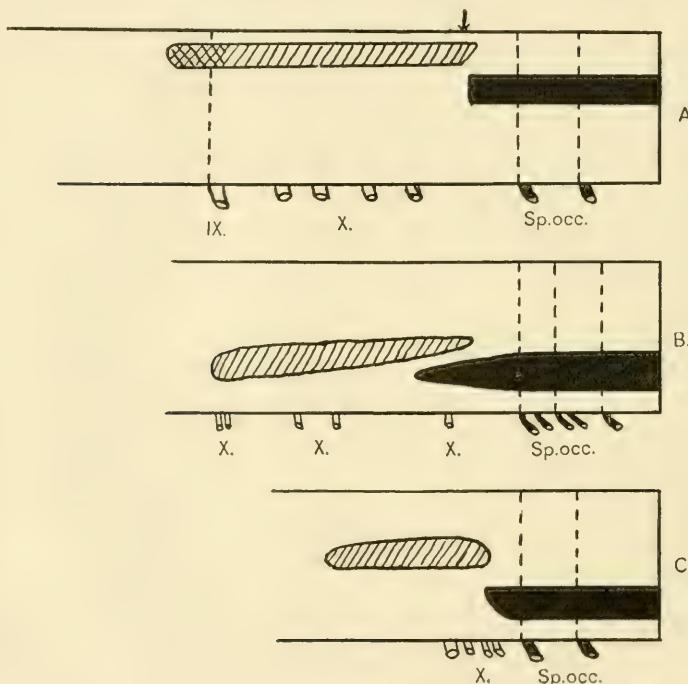
Fig. 8 Reconstruction charts to illustrate the reduction of the rostral end
of the caudal viscero-motor column in Bdellostoma and Myxine. (A), Petro-
myzon fluviatilis (Kappers, 66), (B), Bdellostoma dombeyi, (C), Myxine gluti-
nosa (Röthig and Kappers, 88). Signs and abbreviations as before.

281). In Bdellostoma dombeyi, however, the usual number of
gill sacs is eleven to twelve, though variations both above and
below this are not infrequent (Worthington, 99).

With almost double the number of gills to innervate, it is
not surprising to find the caudal viscero-motor column in Bdellos-
toma almost double the length of the homologous structure in
Myxine. It is, however, difficult to understand why the caudal

viscero-motor column in Bdellostoma should not, for the same reason, be more extensive than the vagus nucleus in Petromyzon also.

Attention has already been drawn by Röthig and Kappers to the apparent 'telescoping' of the brain in Myxine and to the seeming crowding of the vagus rootlets by the otic capsule as a result of such a process (88). Evidences of a similar extensive 'telescoping' in the medulla are not lacking in Bdellostoma. It would appear that the motor and sensory nuclei of the spino-occipital nerves have migrated rostrad, while the superficial attachments of their respective roots have suffered but little disturbance. In other words, the spinal cord appears to be impacted, as it were, into the medulla.

The peripheral area innervated by the spino-occipital nerves is directly contiguous to that supplied by the trigeminus. Their rostral position places the sensory spino-occipital nuclei more directly in connection with the great general cutaneous area of the medulla.

This rostral situation has thus given rise to a close association of the general cutaneous centers of medulla and cord in Bdellostoma, similar in all essentials to that brought about in higher forms by a greater development and specialization of the tractus spinalis nervi trigemini. A different method has been used in the two cases to attain the same result, viz., the close association of centers whose peripheral areas are subject to the influence of simultaneous stimulation.

The rostral projection of the spino-occipital nuclei in front of the exit level of the first spino-occipital nerve appears to be less extensive in Myxine than in Bdellostoma, though it is a curious fact that the distance between the rostral extremity of the nucleus in question and the level of the facial nerve, is approximately equal in the two forms (fig. 7).

In Bdellostoma, on account of the larger size of the vagus column, this nucleus overlaps the spino-occipital nucleus, as already described. However, but little evidence can be adduced to indicate any caudal displacement of the vagus column, the distance between the caudal end of this nucleus and the first

motor spino-occipital root being approximately the same in Bdellostoma and Petromyzon, and but very slightly reduced in Myxine (fig. 8).

With regard to the absence of the glossopharyngeal nerve in Bdellostoma. but little positive evidence can be advanced here. The first (rostral) two vagus rootlets indicated in figures 7 and 8 are so closely associated at both their origin and exit that they should, I believe, be considered as a single root. The probability of this is increased when it is noted that the first vagus root in Myxine is much larger than the succeeding ones. The best evidence on this point, though it be but negative, may be had by reference to figure 8. The reduction of the frontal portion of the caudal viscero-motor column in Bdellostoma when thus compared with Petromyzon is clearly evident, though this reduction is apparently not so extensive as in Myxine.

Such evidence as the above, together with that furnished by the similar observations of Röthig and Kappers (88), tends to confirm Johnston's conclusion that the so-called glossopharyngeal nerve in myxinoids is in reality but a pharyngeal branch of the vagus (53).

With regard to the smallness of the gap between the V–VII nucleus and the vagus column in Bdellostoma, this seems to be the result of a condensation or 'telescoping' of the medulla as a whole, probably in consequence of the complete loss in this form of the peripheral glossopharyngeal area (vide Johnston, 53; Stockard, 90).

Finally, I would point out that the arrangement of the motor roots and nuclei in the medulla of Bdellostoma dombeyi is not a primitive one. On this account I cannot entirely agree with the opinion expressed by Worthington (99, p. 138), and by Ayers and Worthington (4, p. 1) to the effect that myxinoids are the most primitive craniates known and several degrees lower than the Petromyzontes. This conclusion is in harmony with that of Röthig and Kappers based on their study of the motor nuclei in Myxine (l. c.).

SELACHII

Motor nuclei in Selache maxima (Cetorhinus maximus)

The basking shark is the largest of living fishes. It is a pelagic form of wide distribution and often gregarious habit, attaining a length of 35 to 40 feet (Jordan, Bridge, l. c.).

The general morphology of the brain is illustrated in figure 9 and requires no further description in the present connection. Attention may be directed, however, to the marked transverse folding of the large cerebellum. The complexity of the folding
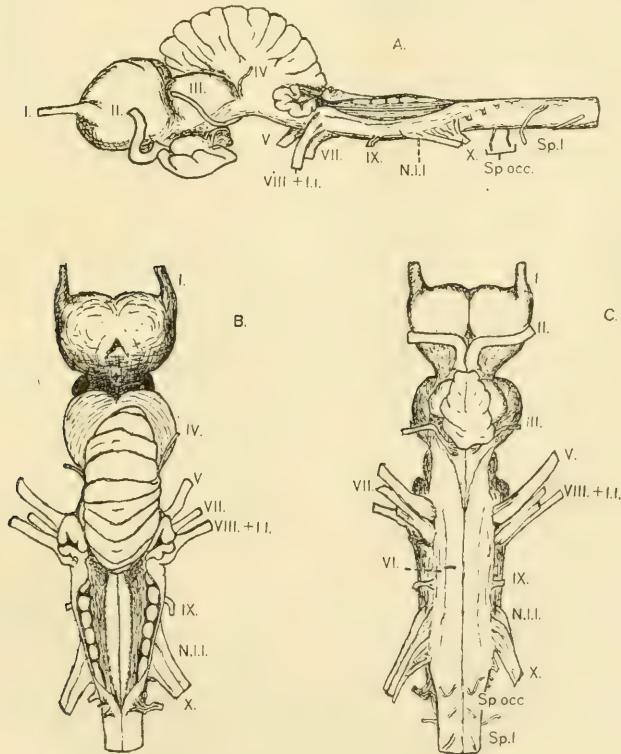
Fig. 9 Brain of Selache maxima. *A*, lateral view; *B*, dorsal view; *C*, ventral view. × ⅔. Abbreviations: *N.l.l.*, nervus lineae lateralis; *sp.occ.*, spino-occipital roots; *Sp.I.*, first spinal nerve; *VIII.+l.l.*, acusticus and lateralis root; *I,II,III,IV,V,VI,VII,IX,X*, cranial nerves.

of the surface of the cerebellum in sharks varies almost directly with the size of the organ in conformity with the general laws of cortical expansion enunciated by Baillarger and modified by Darests (cf. Kappers 68, 69, 71).

Spino-occipital nuclei and roots (*Nu. et rad. mot. Nn. spin. occ.*). Only the first two rootlets of the spino-occipital series are charted in Selache and these probably represent separate fascicles of the first spino-occipital nerve (fig. 17 C). They pass almost directly ventrad from their dorsally placed nucleus of origin to the ventral periphery of the medulla (fig. 10).

As in Bdellostoma, there is no line of demarcation between the anterior horn of the cord and the motor nuclei of the spino-occipital nerves. However, the rostral end of the anterior horn in Selache becomes thinned by the absence of ventrally placed motor cells, so that in the spino-occipital region the somatic motor column in this animal is characterized by a more dorsal position than in the succeeding cervical region. A similar arrangement of the spino-occipital nuclei and roots obtains in all selachians examined. The possible significance of these relations in contrast to those obtaining in ganoids and teleosts will be discussed subsequently.

The rostral limit of the motor spino-occipital nucleus in Selache is sharply marked and the reticular cells of the medulla are not arranged in such evident morphological continuity with this column as in Bdellostoma. The reticular cells form a nucleus whose elements are irregularly distributed in the formatio reticularis from a level somewhat rostrad of the entrance of the trigeminus, downwards to the calamus region. The largest cells tend to be situated centrally and the nucleus corresponds in general arrangement to the reticular nucleus of Raja described by van Hoevell (l. c.). The latter author recognizes three more or less distinct parts to this nucleus, viz., (a) nucleus reticularis inferior which is distributed on the level of entrance of vagus roots, (b), nucleus reticularis medius situated chiefly on the level of entrance of N.VIII, and (c), nucleus reticularis superior distributed over the region rostral to the entrance level of N.VIII.

The arrangement of the reticularis nucleus in Selache and Raja thus corresponds both in its general extent and in the extent of its subdivisions to the nucleus motorius tegmenti described by Bartelmez in Ameiurus (l. c.).
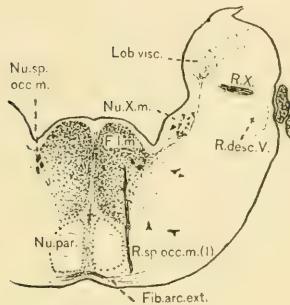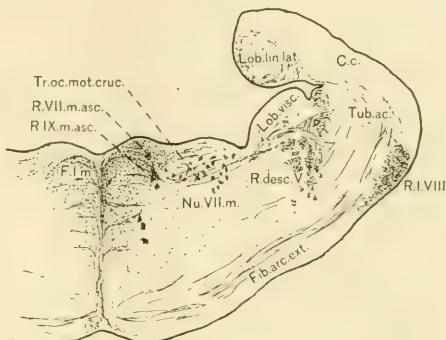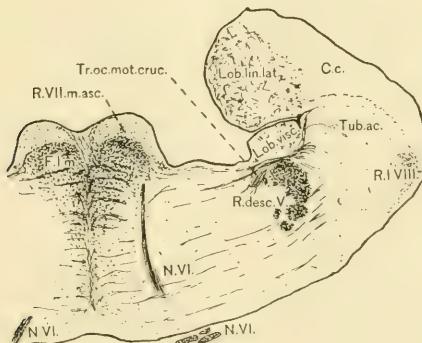
Figs. 10, 11 and 12   Selache maxima.   Outlines of transverse sections to illustrate the topography of the medulla.   Figures 10 to 16 drawn to same scale. Abbreviations: *C.c.*, crista cerebellaris; *F.l.m.*, fasciculus longitudinalis medialis; *Fib.arc.ext.* ventral arcuate fibers of the acusticum; *Lob.lin.lat.*, lobus lineae lateralis; *Lob.visc.*, visceral lobe; *N.VI.* abducens root; *Nu.par.*, nucleus paramedianus (inferior olive); *Nu.sp.occ.m.*, cells of motor spino-occipital nucleus; *Nu.VII.m.*, nucleus motorius N. facialis; *Nu.X.m.*, nucleus motorius N. vagi; *R.desc. V.*, descending sensory trigeminus root; *R.l.VIII.*, root of the posterior lateralis nerve; *R.sp.occ.m.*(1), first spinal-occipital motor root; *R.VII.m.asc.*, ascending facial motor root; *R.IX.m.asc.*, ascending glossopharyngeal motor root; *R.X.*, vagus root; *Tr.oc.mot.cruc.*, tractus octavo-motorius cruciatus; *Tub.ac.*, tuberculum acusticum.

Nucleus paramedianus (*Nu. paramed.*).   The nucleus para-
medianus, or paraseptalis, is well developed in Selache maxima.
The sagittal relations of the right nucleus are indicated in the
reconstruction chart, figure 17 C.   This nucleus is bilaterally
symmetrical, consisting of circumscribed collections of grey mat-
ter, on each side of the raphé near the ventral periphery of the
medulla in the neighborhood of the emergent roots of the
spino-occipital nerves (fig. 10).   The connections which this
nucleus establishes with other centers are not fully understood,
but Johnston has shown that the neurites of this nucleus in
Acipenser cross the raphé and end among the tract cells of the
lateral column (52) and Goronowitsch considered this nucleus
to the homologue of the inferior olive of higher forms (30).   In
general among vertebrates the size and compactness of the
nucleus in question varies directly with the relative develop-
ment of the cerebellum.   This has been pointed out by Kappers,
who has shown that a well circumscribed nucleus paramedianus
is present only in those forms which possess a relatively large
cerebellum (64).

Motor facial, glossopharyngeal and vagal nuclei (*Nu. et. rad.
mot. Nn. VII–IX–X*).   The arrangement of the caudal viscero-
motor column in Selache presents a marked contrast to that
obtaining in cyclostomes (figs. 7 and 17).   In Selache the motor
nucleus of the facial nerve is situated a considerable distance
caudad of the level of its root exit and forms the rostral portion
of a continuous dorsally placed cell column composed of the
motor VII–IX–X nuclei.   This arrangement of the caudal
viscero-motor column is characteristic of all selachians and the
situation of the VII motor nucleus far behind the level of its
own root exit has necessitated the formation of a long ascend-
ing VII motor root in these forms, as already fully set forth by
Kappers (61, 64, 66 and 72).

The relations of this ascending root of the facial nerve in
Selache to its nucleus of origin and to the fasciculus longitu-
dinalis medialis are illustrated in figures 11 and 12.

The motor root of the glossopharyngeus in Selache passes
dorso-mesially from its superficial attachment and courses

through the ventral portion of the VII motor nucleus to reach the lateral side of the posterior longitudinal bundle. Here it turns and courses caudad to its cells of origin in the posterior visceral column lateral to the fasciculis longitudinalis medialis. This mode of origin is essentially similar to that described by Kappers in Scyllium (72, p. 384, fig. 1.).

In Selache and in Hexanchus the ascending motor VII root may be traced caudad as far as the motor glossopharyngeal fibers extend. It results from this that the motor VII nucleus overlaps that of the glossopharyngeus and the two nuclei are quite as intimately associated as are those of the sensory VII and IX roots. This condition is indicated in the reconstruction charts in figure 17 where the caudal limit of the descending VII and IX motor roots is marked by a heavy line.

The motor vagus nucleus in Selache extends for a long distance into the upper part of the cord and in consequence it overlaps the somatic motor column to a much greater degree than was the case in Bdellostoma. Its exact caudal extent was only determined by cutting an additional series of sections of the cervical cord. For comparison the posterior visceral column in Hexanchus was re-examined and it was found the vagus nucleus in this form could indeed be traced further caudad than had been indicated in previous reconstructions, though its exact caudal limit was not determined.

Abducens nucleus and roots (*Nu. et. rad. N. VI*). The abducens nucleus in Selache occupies a position about midway between the exit levels of the motor VII and IX roots. Its cells of origin are scattered among the fibers of the posterior longitudinal bundle and its fibers converge to form three rootlets which pass directly ventrad and emerge in series from the ventral periphery of the bulb (fig. 12). The nucleus in Selache is not quite so dorsally situated as in Hexanchus or Heptanchus, but its relation to the vestibulo-motor fibers is essentially similar in all three forms.

The position of the emergent roots of the abducens at a more caudal level than those of the motor VII nerve is characteristic of sharks and also of ganoids and amphibia. In the case

of Hexanchus both glossopharyngeal and abducens fibers may be cut in the same transverse section (fig. 13).

Motor trigeminal nucleus and root (*Nu. et. rad. mot. N. V.*). The motor trigeminal nucleus in Selache is placed in a dorsal position on the level of its root exit, though it extends some distance both rostrad and caudad of this plane. This situation is a characteristic one for this nucleus in sharks (fig. 17).

In the reconstruction chart figure 17 C, the motor root of the trigeminus in Selache appears to be very small when compared to that of Hexanchus or of Heptanchus. This results from the
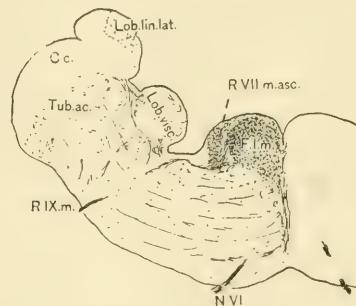
Fig. 13 Hexanchus. Outline of transverse section through the medulla to show the emergence of abducens and motor glossopharyngeal rootlets at the same level. Abbreviations: *N.VI.*, abducens root; *R.IX.m.*, motor rootlet of glossopharyngeus. Other abbreviations as in figures 10 to 12.

fact that the root emerges from the medulla in Selache in a very compact bundle, so that its superficial attachment extends over fewer sections than on the other forms.

The position of the motor V nucleus in transverse section is indicated in figure 14. Here it will be seen that the cells show a distinct tendency to develop processes chiefly in a ventro-lateral direction and the nucleus as a whole is somewhat more ventro-laterally placed than that of the motor VII (fig. 11). In this position the motor V nucleus is placed in close relation to the reticular grey surrounding the upper end of the descending sensory V, and the inconspicuous secondary gustatory tract.

Oculomotor and trochlearis nuclei and roots (Nu. et. rad. Nn. III & IV). The trochlear and oculomotor nuclei in Selache maxima have already been described and charted by Huet (47) and discussed by Kappers (66). However, a short description of these nuclei and their roots will be given here for the sake of completeness.
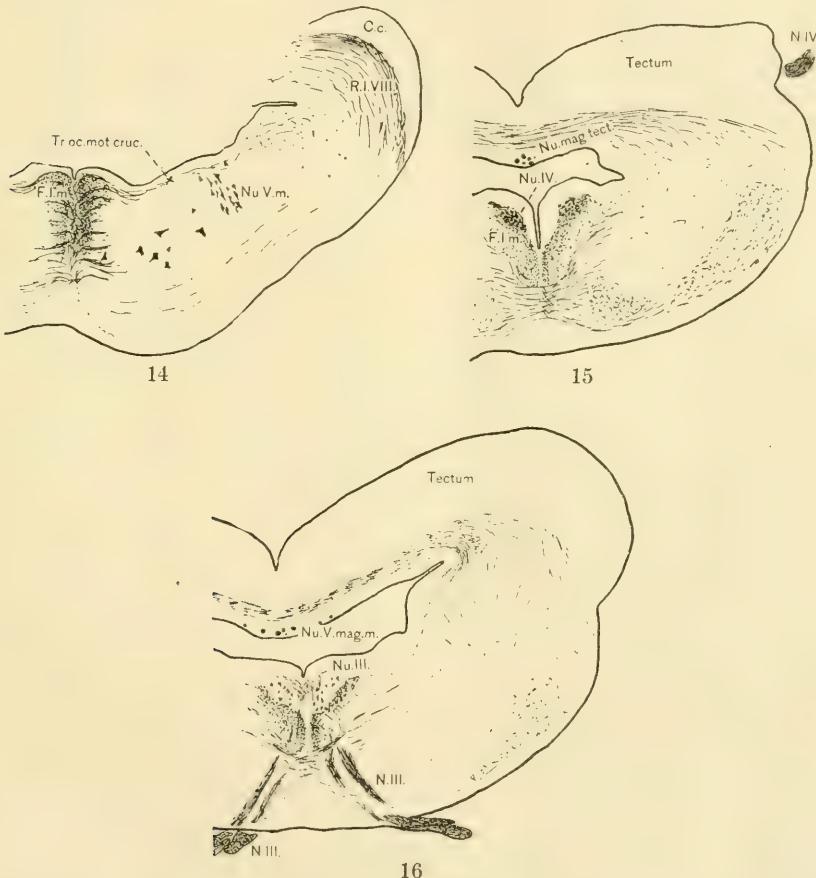
Figs. 14, 15 and 16 Selache maxima. Outlines of transverse sections to illustrate the topography of the medulla. Abbreviations: *N.III.*, oculomotor roots; *N.IV.*, trochlear nerve; *Nu.mag.tect.*, nucleus magnocellularis tecti; *Nu.III.*, oculomotor nucleus; *Nu.IV.*, trochlear nucleus; *Nu.V.m.*, motor trigeminus nucleus. Other abbreviations as in figures 10 to 12.

The trochlear nucleus lies in a crescentic depression upon the dorsal surface of the fasciculis longitudinalis medialis (fig. 15). Its large motor cells are more compactly arranged than those of the oculomotor nucleus with which it is in contact rostrally. The emergent root passes dorsally in the Sylvian gray and, after decussation with its fellow of the opposite side above the ventricle, emerges dorso-laterally between the cerebellum and the midbrain.

The elements composing the oculomotor nucleus are arranged irregularly and are not divisible into definite subsidiary groups (fig. 16). The emergent radicles pierce the posterior longitudinal bundle on their course to the ventral periphery of the midbrain. No definite arrangement of crossed oculomotor fibers could be determined. No small reticular elements such as are characteristically present in higher vertebrates were found in association with either oculomotor or trochlear nuclei.

The sagittal relations of these nuclei and their roots are charted in figure 17 C. The oculomotor nucleus occupies a dorsal position on the level of its root exit and extends rostrad of this level some distance on the same horizontal plane. The trochlear nucleus lies directly behind it but on a slightly more dorsal plane. In most selachians the trochlear root emerges from the brain some distance behind the level of the caudal end of its nucleus (fig. 17 A). In Selache maxima, however, the root emerges at the level of the caudal third of its nucleus (fig. 17 C) and in Hexanchus at the level of the caudal end of its nucleus (fig. 17 B).

Numerous large cells constituting the nucleus magnocellularis tecti may be observed in the ventricular gray of the midbrain roof (figs. 15 and 16). Among the cells of this nucleus, the radix mesencephalica trigemini takes its origin. As this root in all probability is a highly specialized sensory component of the trigeminal nerve, it has not been charted (cf. Johnston, 54; Van Valkenburg, 93 and 94).

Fig. 17   Reconstruction charts of motor roots and nuclei.   (A), Heptanchus (after Kappers).   (B), Hexanchus (mod. after Kappers, 66).   (C), Selache maxima.   Signs and abbreviations as before (page 476).
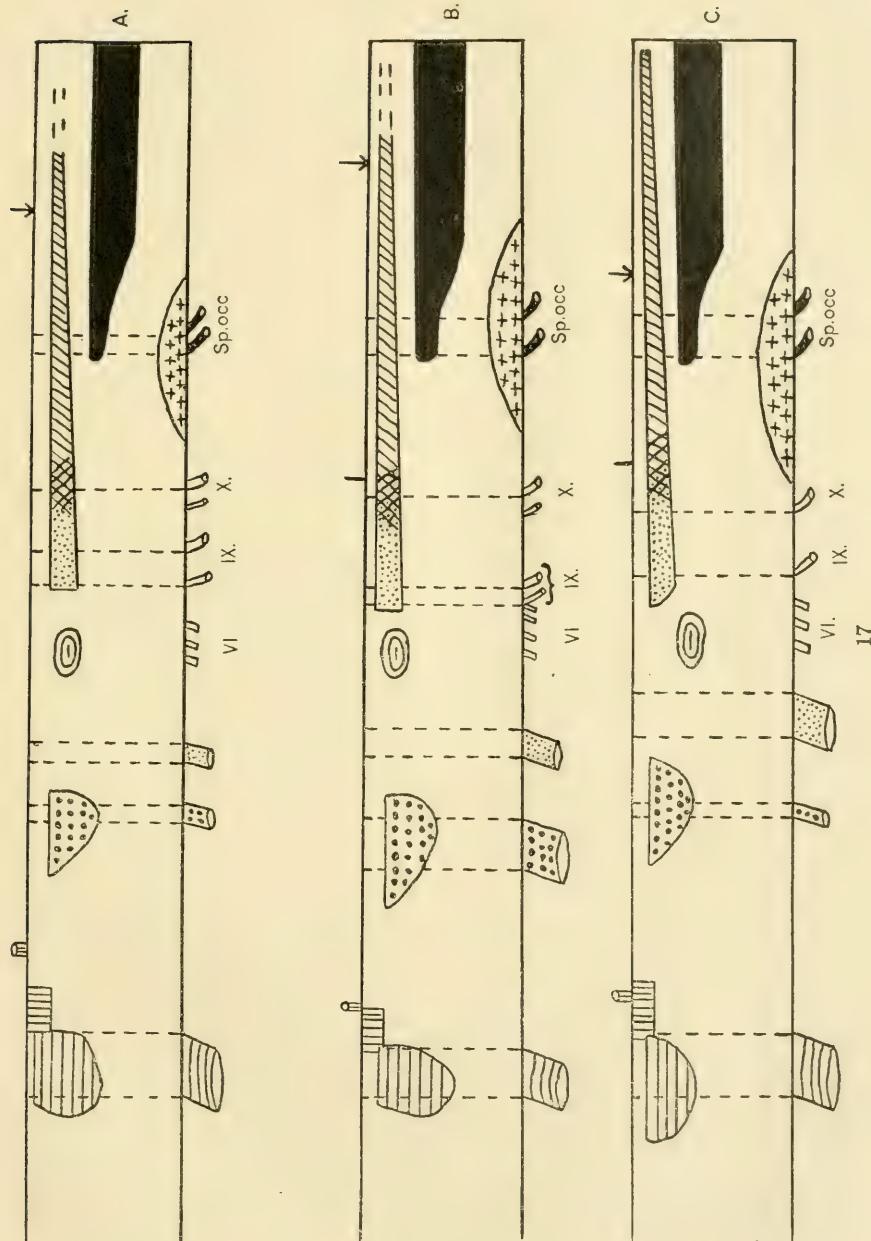
Discussion

Spino-occipital complex. Among sharks the spino-occipital nerves are distributed to both epibranchial and hypobranchial spinal musculature and the pre-hyal elements of the hypo-branchial musculature are well developed (25 and 97). Unlike cyclostomes, there is evidence of a definite specialization of the rostral end of the spino-occipital motor column in sharks, whereby this nucleus becomes dorsally placed, loses its ventral elements, and terminates abruptly a very short distance rostral of the exit level of the first spino-occipital root. In consequence of this, the spino-occipital rootlets in sharks pass from their nucleus of origin almost directly ventrad. These differences between the spino-occipital complex of selachians and that of cyclostomes, are significant in view of Furbringer's findings: that the lower sharks mark a stage in vertebrate evolution where all tendency toward caudal extension of the branchial apparatus comes to an end, so that from sharks onward, there is a definite tendency toward reduction of gill area together with assimilation into the head (and consequent reduction) of cervical segments.

The very evident variation in the number of gills among individuals of Bdellostoma (100) mustexercise no small influence upon the development and stability of the motor center innervating the somatic structures affected by this variation. This would partly explain the lack of any appearance of fixation or specialization of the rostral end of the motor spino-occipital column in these species.

On the other hand, the relative constancy of the development of the pre-hyal coraco-mandibularis elements as well as the epibranchial spinal musculature in sharks (subspinalis and inter-basales, innervated by Furbringer's occipital nerves (v), w, x, y, and z), would tend to produce a relatively constant arrangement of the rostral end of the motor spino-occipital column from which these nerves arise; for progressive reduction of the subspinalis and interbasales among sharks becomes evident only when the higher and lower selachians are compared or when this region is studied ontogenetically and it would thus appear that

in a given species but slight, if any, individual variation in the development of this musculature obtains.

In the absence of disturbing individual peripheral variations it is probable that, as Kappers has pointed out, reflex impulses passing by way of the well developed fibrae arcuatae dorsales and the fasciculus longitudinalis medialis from the acustico-lateral areas, exercise no small influence upon the rostral end of the somatic motor column and contribute largely to the dorsal situation of the cells of this nucleus. Further, the absence of ventrally situated motor elements in the spino-occipital nucleus in selachians would be in accordance with the comparatively slight development in these forms of the ventral reflex pathways, i.e., tractus octavo-motorius cruciatus ventralis, tractus tecto-bulbaris ventralis, etc. (40 and 43).

Motor vagal nucleus (Nu. mot. N. X.). The trapezius muscle in sharks is a well developed structure which receives its innervation by way of the so-called caudal ascending vagus root. This root corresponds to the 'posterior fasciculus' of the vagus which Owen described as "representing the nervus accessorius" (85). This statement has been verified in detail by Furbringer who demonstrated once more the connection between this portion of the vagus and the well developed trapezius muscle in Hexanchus, etc. (25). It follows from this that the caudal end of the motor vagus nucleus must represent the nucleus accessorius which here occupies its most primitive position on the same plane and in undisturbed continuity with the caudal viscero-motor column (fig. 17).

Among sharks, the extent of the viscero-motor column caudad of the exit level of the first spino-occipital rootlets, appears to be more or less directly correlated with the degree of development of the trapezius musculature. Among rays, however, such direct correlation between these structures may not obtain. Thus, according to Kapper's reconstruction (65, fig. 8, 66, fig. 12), the viscero-motor column in Raja clavata extends but a short distance caudad of the exit level of the first spino-occipital nerve. However, this appearance may be due in part to the loss of certain occipital segments which, as Furbringer has shown, are absent among the higher forms of elasmobranchs (rays).

Vetter (95) considers that the trapezius is derived from a primitive superficial visceral constrictor system of muscles which were innervated by the vagus. In sharks, however, the trapezius has lost its visceral constrictor character and, though retaining its vagus innervation, has become a muscle of importance in the somatic movements of the head and pectoral girdle.

The relations of the nucleus accessorius accord well with this hypothesis for, though it retains the essential visceral characteristic of continuity with the caudal viscero-motor column, yet it has come to lie in the upper cervical region where it is closely related to the general somatic sensory centers receiving afferent fibers from the skin area overlying the trapezius. Thus, the peripheral change in muscular function has been accompanied *pari passu* by a central change in the reflex connections of its motor nucleus. In other words, the motor nucleus tends to migrate in the direction of the most important centers acting upon it reflexly.

Motor facial and glossopharyngeal nuclei and roots (Nu. et rad. mot N. VII–IX). The increased importance of the visceral sensibility in the life habits of the selachians, as compared with cyclostomes,[5] is evidenced by the formation in the former animals of a communis nucleus in which the visceral sensory components of the facial nerve terminate in common with those of the glossopharyngeal and vagus nerves and on the level of the latter. The association of the visceral sensory nuclei of these nerves to form a common center situated a considerable distance caudad of the level of entrance of the sensory VII root, affords an example of nuclear association under the influence of similar, simultaneous stimulation (vide supra).

It follows from this, that in selachians, the chief center acting reflexly upon the motor facial nucleus (viz., the nucleus of its own sensory root) is situated at and caudal to the exit level of the glossopharyngeus. As a result the motor VII nucleus, like that of its sensory root, has also become displaced from its

[5] Taste buds among sharks are more numerous than in cyclostomes, though it is probable that they are more or less restricted to the pharynx, gills and mouth in the former animals (vide Kappers, 68).

primitive position on the level of its root exit, and its migration has been in the direction of the greatest number of impulses acting upon it reflexly in accordance with the first concept of neurobiotaxis. The long intramedullary, ascending course over which the emergent motor facial root passes, marks the path along which the nucleus of this nerve travelled during the phylogenetic development of this motor nuclear pattern (v. Kappers, l. c.).

The factors operating to produce the slight displacement of the motor IX nucleus caudal of the level of its root exit, with the resulting formation of its ascending emergent motor root, are essentially similar to those discussed in the preceding paragraph. For further details of the phylogenetic displacements of the motor VII–IX nuclei, reference should be had to Kappers' original papers on this subject (l. c.).

The respiratory mechanism in sharks in general may be described as consisting of two opposing muscular complexes (constrictor group and dilator group) which operate in alternate rhythmic opposition and which are segmentally innervated from before backwards by the motor components of branchial nerves VII, IX, X. The respiratory reflex is initiated chiefly by visceral afferent impulses arising in the branchial mucosa, which reach the communis center through the visceral sensory components of branchial nerves VII–IX–X (6). Thus, the formation of the caudal viscero-motor column by the intimate association of the motor VII–IX–X nuclei and the situation of this column in close relationship with the communis nucleus, furnishes a striking example of the important rôle played by the peripheral respiratory mechanism in the production of specific nuclear (reflex) pattern.[6]

[6] That the primitive segmental nature of the respiratory centers has not become lost by this adjustment is evident from the observations of Hyde (48). This author carried out her experiments on the skate, though unfortunately she has omitted to identify the species of the animals on which she worked. She has demonstrated, however, that the respiratory centers are segmentally arranged and bilateral in nature and has indicated physiologically the characteristic anatomical arrangement of the ascending motor and descending sensory facial roots (l. c., p. 247, fig. 3).

Abducens nucleus and root (Nu. et rad. N. VI). The characteristically dorsal position of this nucleus among sharks appears to be also a primitive one, and in this situation the nucleus is reflexly dominated by impulses from the acustico-lateral area which reach the nucleus through the posterior longitudinal bundle and also directly by dorsal arcuate fibers (64 and 66).

The position of the nucleus between the exit levels of the motor VII and IX roots seems also to be a primitive character, so that the phylogenetic sequence of emergent motor roots in this region evidently should read VII, VI, IX. Indeed in the primitive form Hexanchus the most caudal fascicle of the abducens root emerges on the level of the motor glossopharyngeus exit (vide supra, figs. 13 and 17 B).

Trigeminal nucleus and root (Nu. et rad. N. V.). In all selachians the motor trigeminal nucleus lies in a somewhat dorsal position and extends some distance both caudal and rostral of its root exit. In the latter respect and in its isolation from the motor facial elements, the trigeminal nucleus of sharks differs from that in cyclostomes. Indeed, with the exception of birds, a greater proportion of the motor V nucleus is placed rostrad of the level of its root exit in sharks than in any other group of vertebrates.

In sharks the rostral position of the motor V nucleus, quite removed from the caudal viscero-motor column, is of significance in view of the peculiar arrangement of the respiratory mechanism in these animals. It has been pointed out already that in cyclostomes both inspiration and expiration takes place through the gill openings when the animal is attached by its mouth to any object. As a corollary to this the vascular gill folds in these forms are fixed in such a manner that they can act as efficient respiratory organs without regard to the direction of the flow of water through the gill openings. The gills in sharks consist of closely approximated transverse lamellae which also are firmly attached to the sides of the interbranchial septa. In the latter respect the gills of sharks and cyclostomes resemble one another and differ from those of all other fish.

It is an observation of very long standing (10) that among sharks, during the dilation of the mouth cavity and pharynx in inspiration, water may enter either through the mouth, the spiracle (if present), or even through the gill slits themselves. In sharks, as in cyclostomes, the arrangement of the gills permits of the efficient action of these organs irrespective of the direction of the respiratory flow.[7]

It becomes evident, therefore, that in sharks the alternate opening and closure of the mouth in synchronism respectively with the alternate dilation and constriction of the pharynx, is not a *sine qua non* for efficient breathing. It follows from this that efferent impulses from the motor V nucleus are not necessarily called forth during each reflex respiratory cycle in the same way as are those from the various constituents of the caudal viscero-motor column. The rostral position of the motor V nucleus in sharks may thus be said to express a function of the negative influence of the communis center upon the reflex action of the jaw musculature in these forms.

In this connection the work of Harman (33) on the innervation and development of the musculature of the nictitating membrane and eye-lids in sharks should also be noted. The musculature in question is innervated by the trigeminus and the perfection of its development appears to vary inversely with the size of the spiracle. Thus, according to this author, in rays where the spiracle is of large size, the musculature is absent, while in Galeus where the spiracle is absent or minute, the musculature of the eye-lid is well developed. Scyllium stands intermediate in

---

[7] Baglioni (7 and 8) evidently does not consider that the inflow of water through the external gill openings in selachians is a part of the normal respiratory cycle in these animals. However, Couvreur (14) working on Torpedo marmorata has confirmed this observation and the work of Hyde (48) demonstrates beyond a doubt that respiration of a sufficiently efficient nature to prolong life indefinitely can be maintained in the skate by the action of the last four gill arches alone. This observer also expressly states that the "motor nerves concerned in respiration are the fifth, seventh, ninth and tenth nerves, of which the fifth is least important and the tenth most important" (l. c., p. 240). The relative independence of the trigeminal musculature during the respiratory cycle has also been noted in Rhina by Darbishire (17).

this respect, having a medium sized or small spiracle and a well developed nictitating membrane but no eyelid musculature.

On comparing the reconstruction charts of Raja (66, fig. 12), Scyllium (66, fig. 11) and Galeus (64, map C) it appears that in Galeus the motor V nucleus extends furthest rostral from the frontal margin of the emergent motor VII root, Scyllium comes next in order and in Raja the motor V nucleus reaches nearest to the frontal margin VII root. (For details consult Kappers' list of topographic relations, 64, pages 119–125).

There would thus appear to be some relationship existing between the position of the motor V nucleus in sharks and the degree of development of prespiracular trigeminal eye-lid musculature, though at present no accurate comparison can ,be made.

Though the bulk of the motor V nucleus lies in a dorsal position, there is evidence of a tendency toward ventro-lateral displacement throughout the whole length of the nucleus, for a greater number of the large dendrites of its cells extend ventro-laterally and the diameter of the nucleus itself is increased in this direction (fig. 14).

The elongation of the dendrites and the increased ventro-lateral diameter of the nucleus, bring the motor elements into close relationship with the reticular formation surrounding both the upper end of the descending sensory trigeminal root and the relatively slightly developed secondary ascending gustatory tract.

The influence of the trigeminal cutaneous impulses upon the reflex action of the jaw musculature in elasmobranchs must be great in view of the ventral position of the mouth. In manipulating its food, before this can be swallowed, the animal must rely almost entirely upon its sense of touch. The slight displacement of some of the motor V elements and the extension of processes in the direction of the reticular gray about the rostral end of the descending sensory V nucleus thus illustrate further the first concept of neurobiotaxis (vide supra).

Oculomotor and trochlearis nuclei and roots (Nu. et rad. Nn. III and IV). Compared with petromyzonts, the trochlear nucleus

in sharks is much more rostrally placed and in all the latter forms lies in a dorsal position in the Sylvian gray immediately caudal to the oculomotor nucleus.

In Petromyzon the trochlear nucleus lies on the exit level of the motor V root and caudad of its own root exit, while among sharks this nucleus is always placed far rostrad of the exit level of the motor V root and with few exceptions (e.g., Selache, Hexanchus) among these forms lies wholly rostrad of its own root exit.

The position of the trochlear nucleus just caudad of the oculo-motor nucleus appears to be characteristic of all vertebrates above cyclostomes in which the eyes are functionally well developed. Thus the close mutual association of these two nuclei appears to have occurred phylogenetically at the same time as the development of the midbrain roof to form the great terminal nucleus for the optic tract.

On the other hand, the site at which the trochlear decussation and exit occurs seems to be quite variable even among closely allied forms (cf. teleosts) and it would appear that the root of this nerve, unlike its nucleus, is subject to variable influences of a purely mechanical character.

In conclusion, it is evident that the motor roots and nuclei in Selache maxima are arranged to form a pattern which is in all essentials similar to that obtaining elsewhere among selachians.

In certain respects this selachian pattern exhibits primitive characters, i.e. (a), the emergence of the motor roots in phylogenetic sequence, viz., III, IV, V, VII, VI, IX, X, sp. occ.; (b), the predominantly dorsal situation of both visceral and somatic motor nuclei; (c) the direct continuity and dorsal position of vagus and accessory nuclei, etc. In other respects unmistakable signs of specialization are evident, such, for example, as the relations of the rostral end of the spino-occipital nucleus, the caudad development of the accessory complex, the development of the ascending motor VII and IX roots and the rostral position of the trochlear nucleus in direct contiguity with that of the oculomotor nerve.

GANOIDEI[8]

Motor nuclei in Polyodon spathula

An excellent account of gross features of the brain and of the superficial attachments of the cranial nerves in the Mississippi spoon-bill, has been contributed by Garman (27). Up to the present, however, no work has been published on the finer anatomy of the brain in this animal. Among ganoids, the motor nuclei have already been studied and reconstruction charts have been made in the following forms: Amia (22 and 66), Lepidosteus and Acipenser (91).
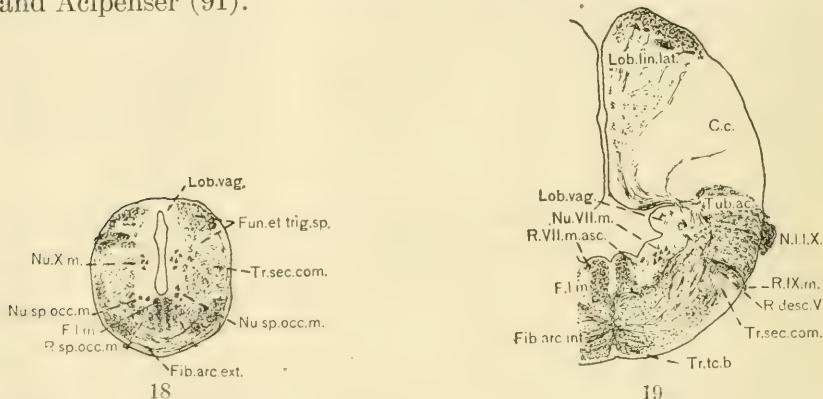
Figs. 18 to 24   Transverse sections of the brain of Polyodon spathula; figures 19 to 24 drawn to the same scale.

Fig. 18   Transverse section of brain stem at the exit level of the first motor spino-occipital rootlet.

Fig. 19   Transverse section of brain stem at the exit level of the motor glossopharyngeus root. Abbreviations: *C.c.*, crista cerebellaris; *F.l.m.*, fasciculus longitudinalis medialis; *Fib.arc.ext.*, external arcuate fibers; *Fib.arc.int.*, internal arcuate fibers; *Fun.et trig.sp.*, funicular area; *Lob.lin.lat.*, lobus lineae lateralis; *Lob.vag.*, visceral sensory column; *N.l.l.X.*, nervus lineae lateralis; *Nu.sp.occ.m.*, motor spino-occipital nucleus; *Nu.VII.m.*, motor facial nucleus; *Nu.X.m.*, motor vagus nucleus; *R.desc.V.*, tractus spinalis trigemini; *R.sp.occ.m.*, first spino-occipital motor rootlet; *R.VII.m.asc.*, ascending bundle of motor facial root; *R.IX.m.*, motor glossopharyngeal root; *Tr.sec.com.*, secondary gustatory bundle; *Tr.tc.b.*, tractus tecto-bulbaris; *Tub.ac.*, tuberculum acusticum.

[8] This term is retained because of its great convenience and is employed here in the sense defined by Jordan (55, p. 32). With regard to the literature on Polyodon, I am much indebted to Professor C. J. Herrick both for the loan of Garman's paper (27) and for assistance during my vain search for any work dealing with the finer anatomy of the brain stem of this animal.

Spino-occipital nuclei and roots (Nu. et rad. mot. Nn. spin. occ.).
The dorsal portion of the somatic motor column of the cord, is
continued upwards into the medulla in Polyodon in a manner in
many respects recalling the relations prevailing in this region
among selachians (figs. 10 and 18). The rostral end of this
column is, however, not sharply defined as in sharks, and it
is difficult to set a limit between the nucleus in question and the
reticular elements of higher levels. A similar condition was
noted in Bdellostoma. In figure 25 A, the level above which
none of the emergent motor rootlets can be traced is taken to
be the rostral limit of this motor column.

Like the spino-occipital column in Selache, this nucleus in
Polyodon lies close to the ventricular gray, lateral to the fasciculus
longitudinalis medialis, and the cells toward its rostral end are
most dorsally placed. The emergent motor fibers pass very
obliquely ventrad and caudad from this nucleus to reach the
periphery of the bulb. In the latter respect these roots differ
from the emergent spino-occipital fibers in Selache which pass
almost directly ventrad (figs. 10 and 18). It should also be
noted as a corollary to this observation, that in none of the
selachians hitherto examined, does the spino-occipital nucleus
extend so far rostrad of the first spino-occipital rootlet as does
this column in Polyodon, above its first emergent fiber. Of the
ganoids examined, Acipenser only seems to be an exception to
this rule (figs. 17 and 25). The origin and relation of the spino-
occipital nerves in Amia and Lepidosteus, and in Acipenser
(except as noted above) closely correspond to those obtaining in
Polyodon.

Nucleus paramedianus (Nu. paramed.). On each side of the
raphé, on the level of the first spino-occipital rootlet and extend-
ing for some distance caudad, the nucleus paramedianus may be
distinguished in Polyodon. Many internal arcuate fibers course
across this ill defined nucleus, whose cells, as in Amia, are so
diffusely distributed that it was not possible to indicate its out-
line in the reconstruction chart. However, in both Acipenser
and the young Lepidosteus specimens which Theunissen exam-
ined, the nucleus paramedianus has been charted.

Motor facial, glossopharyngeal and vagal nuclei and roots (Nu. et rad. mot. Nn. VII–IX–X). In Polyodon the caudal visceromotor column lies throughout within the ventricular gray and extends from a level about midway between the emergent motor roots of the facial and glossopharyngeal nerves, to a point some distance caudad of the first spino-occipital motor rootlet (figs. 18, 19 and 25 A). As in Selache, within the limits of this column are included all the cells of origin of the motor VII–IX–X roots.
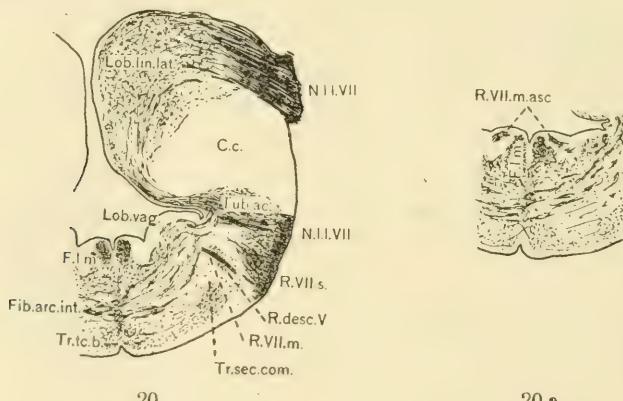
20

20 a

Fig. 20 Polyodon spathula. Transverse section of brain stem at the exit level of motor facial root.

Fig. 20 a Portion of transverse section of brain stem at somewhat more caudal level than the preceding to illustrate the relations of the ascending motor facial root. Abbreviations: *N.l.l.VII.*, nervus lineae lateralis; *R.VII.m.*, emergent fibers of motor facial root; *R.VII.m.asc.*, ascending bundle of motor facial root; *R.VII.s.*, sensory facial root. Other abbreviations as in figures 18 and 19.

The fibers of the motor VII root arise from cells occupying the rostral end of the caudal visceral motor column. They collect to form a compact bundle which courses rostrad for a considerable distance in the ventricular gray beside the fasciculus longitudinalis medialis, before they arch laterally and emerge on the ventro-lateral periphery of the medulla (figs. 19, 20 and 20 A).

The glossopharyngeus motor root arises from cells of the viscero-motor column situated immediately caudad of the motor

VII nucleus. The fibers pass rostrad a short distance and form a root which courses laterad below the motor VII nucleus to gain the ventro-lateral periphery (fig. 19).

The arrangement and relations of the constituents of the caudal visceral motor column and their motor roots in Acipenser, Amia, and Lepidosteus, are essentially similar to those described above in Polyodon.

Abducens nucleus and roots (*Nu. et rad. N. VI*). In the tegmentum, medial and somewhat ventral to the rostral end of the motor VII nucleus, a poorly defined and scattered collection of cells forms the abducens nucleus. In Polyodon, as in Lepidosteus, the presence of numerous reticular elements in this neighborhood makes it difficult to define the exact limits of this small nucleus. Thus, in Polyodon it is only possible to indicate the approximate outline of the abducens nucleus by means of dotted lines. Its rostro-caudal extent in figure 25 represents the limits beyond which no emergent radicles could be followed centrally. In studying Lepidosteus, Theunissen also experienced this difficulty so that, though he described the nucleus in question, he has not indicated it in his reconstruction chart (fig. 25 D). In Amia and Acipenser, however, the abducens nucleus is somewhat less diffusely arranged and its position is indicated in the reconstruction charts (fig. 25 C and B).

Three thread-like rootlets emerge in series from the abducens nucleus in both Polyodon and Acipenser, while four such rootlets can be identified in Lepidosteus and Amia. In each case they course almost directly ventrad to the periphery of the bulb.

The position of the emerging abducens rootlets in ganoids midway between the exit levels of the motor VII and IX roots, corresponds closely to the arrangement of these roots in sharks. Also in regard to the position of the abducens nucleus with reference to the exit level of the motor glossopharyngeus, the two groups closely resemble one another. In sharks, however, the abducens nucleus lies upon a more dorsal plane than it does in ganoids.

Motor trigeminal nucleus and roots (Nu. et rad. mot. V). In Polyodon, the motor V nucleus is dorsally situated in the lateral area of the ventricular gray, and it lies wholly caudal to the rostral border of its emerging root (fig. 25 A). In the rostral third of its length the nucleus becomes thinned somewhat, so that a partial division into two is evident. The fibers arising in this nucleus become collected to form a bundle which ascends just laterad of the nuclear mass (fig. 21 A). Toward the
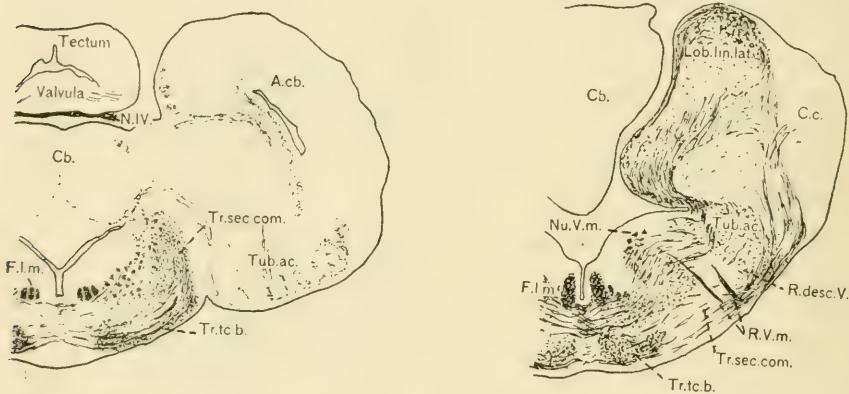
22   21
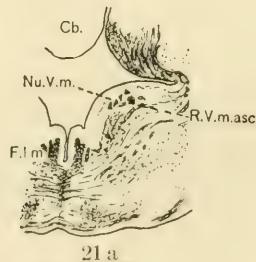
21 a

Fig. 21 Polyodon spathula. Transverse section of brain stem at the exit level of the motor trigeminus root.

Fig. 21á Portion of transverse section at somewhat more caudal level than the preceding to illustrate the relations of the ascending motor trigeminal root.

Fig. 22 Transverse section of brain stem at the exit level of the trochlear nerve. Abbreviations: *A.cb.*, auricular lobe of cerebellum; *Cb.*, cerebellum; *N.IV.*, trochlear nerves decussating at their emergence; *Nu.V.m.*, motor trigeminal nucleus; *R.V.m.*, emergent motor trigeminal fibers collected into two rootlets; *R.V.m.asc.*, ascending fibers of motor trigeminal root. Other abbreviations as before.

rostral end of the nucleus, the bundle curves ventro-laterad to reach the periphery. In this part of its course the fibers become arranged in the form of two distinct emergent bundles, one more rostrally placed than the other. These rootlets are separated only by a few sections, and it was not possible to demonstrate any specific localized relationship between the two rootlets on the one hand, and the two nuclear subdivisions on the other; each rootlet apparently derives its fibers from the nucleus as a whole, and not from any special portion of it.

Aside from the partial division of the motor V nucleus and the double nature of its emergent root, the relations of this complex in Polyodon correspond closely to those in Lepidosteus (fig. 25 A and C). In all other ganoids examined, the nucleus in question is dorsally situated and its chief bulk is placed caudad of the level of its root entrance. The last mentioned relation is especially evident in Amia, where the nucleus extends caudally well beyond the level of the emergent motor VII root. In selachians, on the other hand, the motor V nucleus is placed more nearly on the level of its root entrance and, while it always extends a considerable distance rostrad of this point, the nucleus never extends caudad of the exit level of the motor VII root (fig. 17). Among cyclostomes, however, the motor V nucleus in Petromyzon closely corresponds to that of Polyodon both in its position and its extent. Similarly in Myxine and Bdellostoma, the motor V nucleus agrees with that of Polyodon both in its rostro-caudal extent and in its relation to the exit levels of the motor V and VII roots, though their resemblance is somewhat obscured owing to the very ventral situation of the nucleus in question and its continuity with motor VII column in the former animals (figs. 7, 17 and 25).

Oculomotor and trochlear nuclei and roots (*Nu. et rad. Nn. III and IV*. The oculomotor and trochlear nuclei are both small in Polyodon, as in other ganoids. The oculomotor nucleus lies upon the dorso-median aspect of the fasciculus longitudinalis medialis in the Sylvian gray and is placed entirely rostrad of the caudal border of its emergent root (fig. 25 A).

It is possible to distinguish two cell groups within the nucleus: a dorsal group which is present throughout the nucleus, and a group more ventrally situated which is present only in its caudal portion. In figure 24, some cells of the latter group may be seen lying ventrally, between the fasciculi longitudinales mediales and below the small dorsal cell groups. The emergent rootlets pass ventrad through the fine strands of the commissura ansulata.
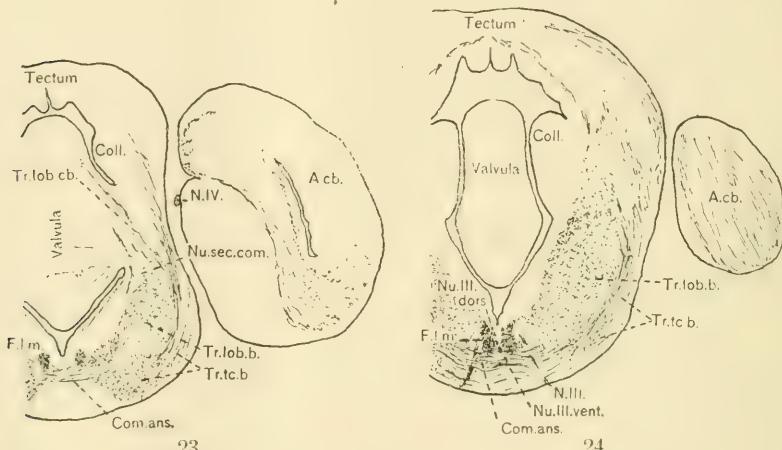
Fig. 23 Polyodon spathula. Transverse section of brain stem to illustrate the relations of the trochlear nerve to the neighboring structures.

Fig. 24 Outlines of transverse section of brain stem at exit level of oculomotor root. Abbreviations: *Coll.*, colliculus; *Com.ans.*, commissura ansulata; *N.III.*, emergent bundles of oculomotor nerve; *N.IV.*, trochlear nerve; *Nu.III.dors.*, dorsal moiety of oculomotor nucleus; *Nu.III.vent.*, ventral moiety of the oculomotor nucleus; *Tr.lob.b.*, tractus lobo-bulbaris; *Tr.lob.cb.*, tractus lobo-cerebellaris. Other abbreviations as before.

In both Amia and Acipenser, dorsal and ventral cell groups may be distinguished in the oculomotor nucleus, though in the young Lepidosteus specimens described by Theunissen this condition was not apparent. Among these forms the oculomotor complex occupies the most rostral position in Amia, where the nucleus lies almost wholly in front of the rostral border of its emergent root. In Acipenser the nucleus is somewhat more caudally placed while in both Polyodon and Lepidosteus the

caudal end of the nucleus corresponds to the level of the caudal border of the oculomotor root (fig. 25). In Polyodon the sub-division of the elements of the small oculomotor complex into dorsal and ventral cell groups is unmistakable and presents an interesting contrast to the conditions obtaining in the large undifferentiated oculomotor nuclei in Selache.

The trochlear nucleus in Polyodon is placed some distance caudal to the oculomotor nucleus. Its relation to the fasciculus longitudinalis medialis is essentially similar to that of the dorsal cell group of the oculomotor complex to the same structure. From the trochlear nucleus a very fine strand of fibers arises. In its caudal course the root passes dorso-laterally through the ventricular gray into the valvula. Immediately beneath the floor of the crevice between the valvula and the cerebellum, the nerves of either side decussate and emerge from the dorsal surface of the brain in the mid-line. The peculiar transverse band which the two trochlear roots form at their decussation between the valvula and the cerebellum, is shown in figure 22. Garman (1. c.) has drawn attention to the similarity of the trochlear exit in Acipenser and Polyodon. The nerve roots course laterad after their exit and then pass rostrad between the auricular lobes of the cerebellum and the tectum (fig. 23).

In Polyodon, Acipenser and Lepidosteus the position of the trochlear nucleus with reference to its root exit and that of the oculomotor nerve is almost identical. In Amia, however, in correspondence with the more rostral position of the oculomotor nucleus, that of the trochlear nerve is placed nearer the level of the caudal border of the oculomotor root than in other ganoids (fig. 25).

Discussion

Spino-occipital complex. In ganoids there are no epibranchial spinal muscles as in sharks (97) and the pre-hyal hypobranchial elements are represented by the musculus branchio-mandibu-laris. Lepidosteus, however, would appear to be an exception to the latter part of this statement for according to Edgeworth (20) there is no trace of the m. branchio-mandibularis (m. genio-
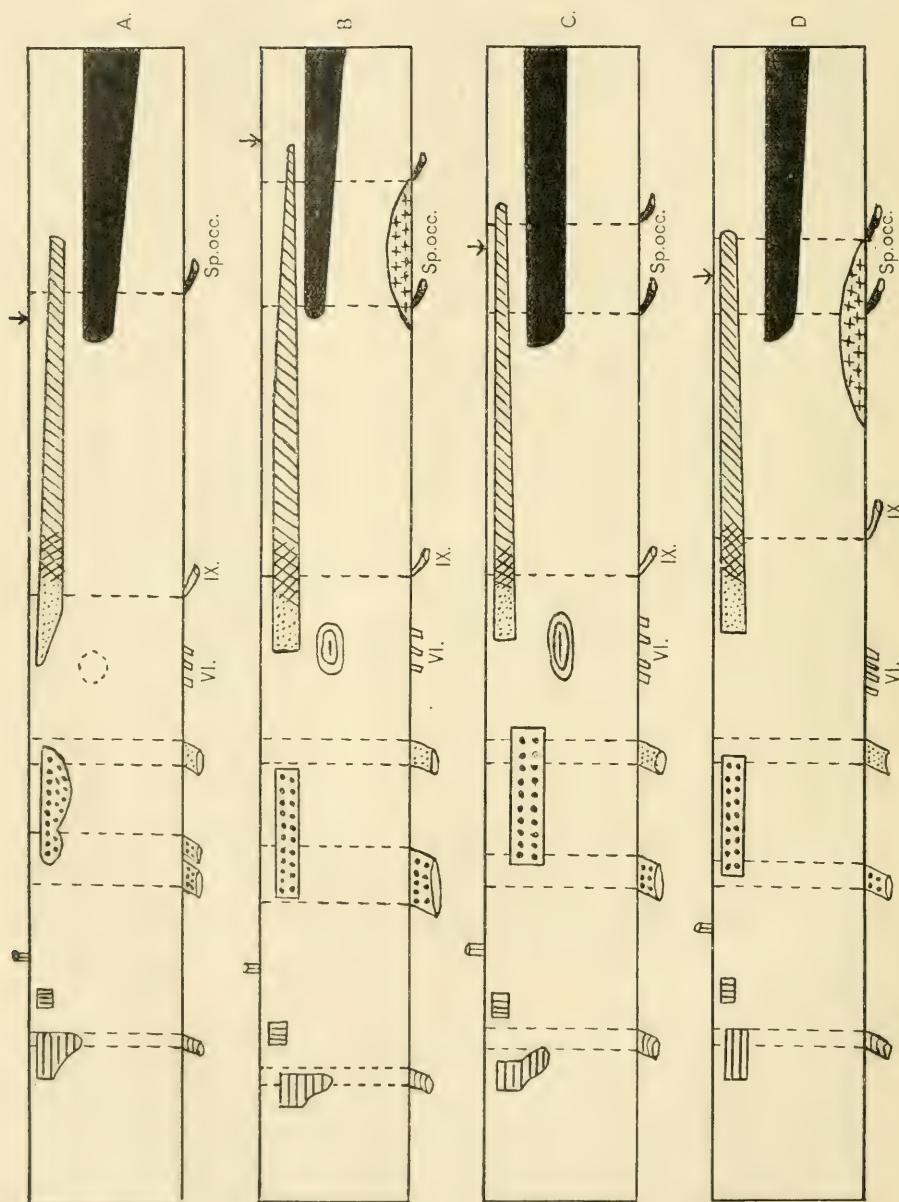
Fig. 25 Reconstruction charts of motor roots and nuclei. V. Polyodon, B. Acipenser (after Theunissen, 91), C. Lepidosteus (after Theunissen, 91), D. Amia (after Kappers, 66). Signs and abbreviations as before (vide p. 476).

hyoideus) to be found even in the embryo of this form. In Amia according to Allis (1) this muscle, which is subject to great variation in different individuals, "undoubtedly represents a muscle in the process of deterioration and disappearance." This author demonstrated that the nerve supply of the branchio-mandibularis is derived from a terminal twig of the fused ventral branches of the first, second and third occipital nerves (equivalent to Furbringer's nerves z, a and b). McMurrich (83), though unable to trace the innervation of this muscle in Amia, pointed out that it was the probable forerunner of the muscles of the tongue of higher forms, and that, as no trace of the branchio-mandibularis is to be found in teleosts, Amia was probably the last piscine form to possess it. This muscle was found in Acipenser by Vetter (95), in Polypterus by Pollard (86) and Edgeworth (l. c.) and in Polyodon by Danforth (16). In the latter animal the branchio-mandibularis is a very small muscle whose innervation was not actually determined but whose relations, when compared with Amia, left no doubt as to its complete homology with the similarly named muscle in that form.

In correspondence with the evident reduction of these peripheral elements in ganoids as compared with sharks, a reduction in the number of spino-occipital nerves has occurred in the former group. The most rostral of these nerves found in the ganoids is Furbringer's occipital nerve x which occurs in individual cases in Acipenser alone, while the occipital nerve z is the only one of this sub-group of nerves which is characteristically retained in all ganoids.

Within the central nervous system, one expression of these peripheral changes is to be seen in the oblique caudal course which the emergent spino-occipital rootlets take from their nucleus to the periphery—as if the somatic motor column were pushed rostrad, dragging with it the remaining motor rootlets. Acipenser alone proves an exception to this rule and according to Furbringer it is just this form among ganoids that shows the least reduction in the number of occipital nerves. It is also significant to note in this connection that alone among ganoids, coraco-

branchiales muscles (which, as is sharks, are innervated by spino-occipital nerves), are developed from all the branchial myotomes in Acipenser (Edgeworth, Vetter, Furbringer).

That a rostral migration of the spino-occipital nucleus should occur *pari passu* with the reduction and absorption into the head of occipital elements, is rather to be expected and further evidence of such a displacement of this nucleus is not lacking. Thus, if the distance between the first emergent spino-occipital root and the emergent motor IX nerve be measured in sharks and ganoids (figs. 17 and 25), a comparison will show this distance to be the same in Lepidosteus and Selache and almost the same in Acipenser, Amia and Hexanchus, though a greater discrepancy is evident in the case of Polyodon. In other words, though certain occipital nerves are known to have been lost in ganoids, those that remain have been displaced rostrad.

Rostral displacement of the spino-occipital column must have been preceded first by the loss of its peripheral motor elements (motor perikaryons), followed by the reduction and modification of the remaining coordination elements of the nucleus. This is indicated in ganoids as in Bdellostoma by the difficulty with which the rostral end of this nucleus is defined. The column passes over gradually into the inferior reticular nucleus and thus appears to extend a considerable distance further rostrad of the exit level of its first root than is really the case (vide supra).

It should also be noted that the peripheral pre-hyal hypobranchial musculature, at least in Amia, shows evidence of considerable individual variation, so that a condition may obtain here somewhat analogous to that already pointed out in Bdellostoma, where the individual variation was so great that a question arose as to whether the extent of the somatic motor column in the reconstruction chart in figure 7 could be taken as representative of the species.

Aside from this difficulty of delimitation, the rostral end of the somatic motor column in ganoids shows unmistakable evidence of specialization, so that it differs considerably from the anterior horn in the cervical region. The rostral end of the

spino-occipital nucleus is situated dorsally upon the fasciculus longitudinalis medialis, and, as in sharks, is lacking in ventral elements. This position is probably due to the same influences that were noted in the discussion of this region in Selache, viz., to the action of reflex impulses by way of the well developed dorsal arcuate fibers and posterior longitudinal bundles and to the comparatively slight development of ventrally situated reflex pathways.

Allis (l. c.) has suggested that "the tongue of the adult Amia may, therefore represent a condition of that organ in which its partial muscularization has been lost, rather than not yet acquired, as Gegenbauer (28) states to be the case for fishes in general." However, within the central nervous system, no evidence has yet been forthcoming to indicate that the spino-occipital nucleus in Polyodon or other ganoid was at any time more specialized than is this complex in modern sharks. But it is interesting to note that in both sharks and ganoids the nucleus which innervates the forerunner of the tongue musculature is placed, as in higher forms, in close proximity to the visceral motor center innervating the musculature of the foregut.

Nucleus paramedianus (Nu. paramed.). The varying development of the nucleus paramedianus among ganoids is worthy of note in connection with the question of the homology of this nucleus and the inferior olive of mammals. In Amia, which probably represents the nearest relative of the modern teleost group, there is no well circumscribed nucleus paramedianus and, since this nucleus does not appear as a definitely circumscribed gray mass in teleosts, amphibians or reptiles, it is possible and even probable that the nucleus in question was represented in the ganoid stock merely by an undifferentiated reticular area. Thus, the nucleus paramedianus of selachians would appear to be a structure independently specialized within the group after its divergence from an ancestral type common to sharks and ganoid stock.

In Polyodon, the acusticum and lobus lineae lateralis are directly continuous with the cerebellum, with which they corre-

spond in structural detail, as Johnston (49) long ago pointed out
to be the case in Acipenser. The acusticum and lobus lineae
lateralis in Polyodon are relatively even larger than in Acipenser
and together with the cerebellum form by far the greater bulk
of the rhombencephalon. It is possible that the poorly circum-
scribed but recognizable nucleus paramedianus in Polyodon and
in other ganoids has been differentiated under the influence of
these structures and does not represent the more or less degen-
erated remains of a once more highly developed nucleus. By
this it is implied that the acusticum, lobus lineae lateralis and
cerebellum together in ganoids bear the same causal relation-
ship to the differentiation of the paramedian area as does the
cerebellum to this area within the groups in which the latter
organ has independently gained a high state of relative speciali-
zation, viz., sharks, birds, and mammals.

Motor vagus nucleus (*Nu. mot. N. X.*). The trapezius muscle
in ganoids has become much reduced as compared to selachians,
a condition which is probably due in a large measure to the
development of the operculum and consequent general modifi-
cation of the musculature in the branchial region.

In Acipenser the trapezius is represented in the 11 mm. em-
bryo (Edgeworth) but, according to Vetter, disappears in the
adult. In Amia, Lepidosteus, and Polypterus, Edgeworth de-
scribes the development of the trapezius. In the first named
form this muscle, which is described by Allis (l. c., p. 669) as
the 'fifth externus' levator of the branchial arches, is inner-
vated by a branch of the vagus. In Polyodon also the trapezius is
present though not a large muscle, and is innervated by a long
branch of the vagus (Danforth).

It is to be expected from what has been said in the case of
selachians that a reduction of the trapezius musculature such as
obtains in ganoids would lead to some curtailment in the caudal
extent of the vagus nucleus. Judging from a comparison of the
extent of the overlap of the caudal viscero-motor column and
spino-occipital nucleus in the selachians and ganoids charted
(figs. 17 and 25), some shortening of the vagus nucleus in the
latter forms is apparent. However, as a greater overlap of these

two nuclei obtains in the selachians charted in figure 17 than in many other sharks hitherto examined (vide 66, 72, etc.) and since it is known that ganoids have lost certain occipital nerves which are characteristically present on sharks, but little if any reliance can be placed upon the results of such a direct comparison as that suggested above.

A comparison of this region among ganoids themselves (fig. 25) brings out the fact that the extent of the vagus column caudad of the exit level of the first spino-occipital rootlet, as well as the extent of overlap of the caudal vagus column and spino-occipital nucleus, is greatest in Acipenser and least in Polyodon, while in these respects Amia and Lepidosteus occupy an intermediate position in the series. An examination of the relative development of the musculature to which the vagus is distributed shows that these central relations closely agree with the peripheral conditions obtaining in these forms (cf. discussion of spino-occipital complex).

Thus, though the trapezius be absent in Acipenser, the levatores arcuum branchialium externi which are closely related to this muscle ontogenetically, are more numerous in this form than in Amia (McMurrich, l. c.) and they are chiefly innervated from the caudal vagus column. Further, in Amia (Allis) and in Lepidosteus (Edgeworth) in both of which the trapezius is a very small muscle, the only coraco-branchialis present in these forms (pharyngo-clavicularis externus) receives its innervation from the caudal part of the vagus (cf. supra, innervation of coraco-branchiales in Acipenser). Finally in Polyodon, where a reduction of the caudal part of the vagus nucleus appears to be most evident among ganoids, the pharyngo-clavicularis is innervated not by the vagus but by the first spinal (Danforth),[9] the trapezius though present is small, and the levators of the branchial arches are not so extensive or so numerous as in Acipenser.

[9] If the so-called pharyngo-claviculares muscles of Polyodon are homologous with the coraco-branchiales muscles of Acipenser, as their respective nerve supply would suggest, then in this respect both these forms resemble selachians, while in Amia and Lepidosteus, on the other hand, the pharyngo-claviculares muscles receive their innervation in a manner similar to that obtaining among most teleosts.

From the above it becomes evident that, though the caudal portion of the motor vagus nucleus is well formed in ganoids, yet in these animals the elements homologous with those forming the bulk of the accessory nucleus of higher forms must necessarily be few. It is also evident that, apart from the question of the homology of the muscles whose innervation has been noted with similarly named muscles in other forms (Herrick, 34) the variations in peripheral motor distribution in this region within the ganoid group appear to be faithfully reflected in the arrangement of the central motor nuclei.

Motor facial and glossopharyngeal nuclei and roots (*Nu. et rad. mot. Nn. VII–IX*). The arrangement of the motor nuclei forming the rostral part of the caudal viscero-motor column in ganoids is, *inter se*, typically selachian, consisting as it does of a nuclear mass from which arise the motor facial and glossopharyngeal roots and which is continuous caudally with the motor vagus nucleus. There is, however, a significant difference to be observed in the relations to neighbouring structures of this part of the caudal viscero-motor column as a whole in the two groups.

In every case among ganoids the chief bulk of the elements forming the motor facial nucleus, lies rostrad of the exit level of the motor IX root. In selachians, on the other hand (at least among the more primitive members of the group) the reverse is true and the motor VII nucleus lies chiefly caudad of the exit level of the motor IX root.

The different position of the motor VII nucleus with reference to the exit level of the motor glossopharyngeus in these two groups might of course be due to a shifting of the motor IX root. However, the relation of the nucleus in question to that of the abducens nerve and to its root in both ganoids and sharks would tend to negative such a supposition.

It has been pointed out already that the position of the abducens nucleus with reference to the exit level of the motor glossopharyngeus, is practically the same in both ganoids and sharks, so that for purposes of comparison here it is reasonable to consider this nucleus as a more or less fixed point.

The entire motor VII nucleus in all sharks lies characteristically some distance caudad of the abducens nucleus and the rostral end of the motor facial nucleus is placed either caudal to or at the level of the last emergent abducens root. On the other hand, in all ganoids the rostral end of the motor VII nucleus overlaps the abducens nucleus and its emergent rootlets for a considerable distance (figs. 17 and 25). It thus becomes apparent that the motor VII nucleus of ganoids lies on a more rostral level in the medulla than does this nucleus in sharks. In this respect ganoids resemble teleosts (vide infra).

In the discussion of this region in selachians, the important part played in the formation of the ascending motor root of the facial and glossopharyngeal nerves by the development in these animals of a caudally situated communis nucleus, has already been indicated. In Polyodon the communis system seems not to be so highly developed as in sharks but to be overshadowed in relative importance by the large and extensive acusticum and lateral line area (figs. 11 and 13 with 19 and 20). As a somewhat similar condition obtains in Lepidosteus and Acipenser, this may account in part at least for the less caudal position of the motor VII nucleus in these forms than in sharks. This question is considered further in the discussion of the motor V nucleus (vide infra).

In this connection it may be noted that the structure of the brain stem of Polyodon furnishes no evidence indicative of a gustatory function for the 'primitive pore' elements which are so numerous on the head and bill of this animal (Kistler, 76; Collinge, 13). On the other hand, the highly specialized and extensive acusticum and lobus lineae lateralis in Polyodon furnish strong presumptive evidence in favor of these peripheral organs being of the functional nature of neuromasts. As Wright (102) originally employed this term to distinguish pear-shaped hair cells from rod-shaped taste cells, and since the sense-cells of Kistler's figures strongly resemble the latter elements, the term neuromast is here used in a strictly functional sense, i.e., to indicate organs centrally related by afferent nerves to the special somatic area of the rhombencephalon.

Abducens nucleus and roots (Nu. et rad. N. VI). The position of the poorly defined abducens nucleus in all ganoids midway between the exit levels of the motor VII and IX roots, is determined in all probability by the termination of the acoustic nerve at this level. Kappers (64) has already pointed out the importance of this factor as well as that of the dorsal arcuate fibers of both the crossed and direct octavo-motor tracts in determining the position of the abducens nucleus in sharks. The nucleus in Polyodon is small in correspondence with the slight functional development of the eye and lies somewhat ventrally in the tegmentum between the tractus tecto-bulbaris and the fasciculus longitudinalis medialis and is traversed by the arcuate fibers of the tractus octavo-motorius.

In all ganoids the abducens is placed more ventrally in the tegmentum than in sharks and appears to be characteristically more closely associated with the tractus tecto-bulbaris in the former animals. This apparently would indicate that the tractus tecto-bulbaris was more highly developed in ganoid ancestors than in the modern representatives of the group, for otherwise it is difficult to account for this ventral position of the abducens nucleus in the presence of such evidently well developed dorsal acustico-motor tracts.

Such a conclusion is strengthened by the relations obtaining in the oculomotor and trochlear nuclei in Polyodon to be noted subsequently and also by the findings of Danforth (l. c.) in his dissections of the eye-muscles in this form. According to this author, considerable variation was met with in connection with these muscles and in one case he records the complete suppression of the external rectus on the left side as evidence of a retrograde tendency in this area. Further, Allis (l. c.) regards the type of arrangement of the eye-muscles in ganoids as more specialized than in selachians, and, though this generalization may be subject to some question, yet the fact remains that, like Polyodon, in Amia the external rectus muscle also varies in its development.

Motor trigeminal nucleus and root (Nu. et rad. mot. N. V). In all ganoids the motor V nucleus occupies a dorsal position in the

ventricular gray and is placed wholly caudad of the rostral border of its emergent root. In its dorsal position this nucleus resembles that of sharks, but in its extent caudad of its emergent root the nucleus recalls the condition obtaining in cyclostomes and, in a manner of speaking, it foreshadows the more complicated relations which are characteristic of the motor V nucleus among teleosts.

It is an unfortunate fact that the mechanism of branchial respiration has not been investigated in ganoids, although the air-breathing propensities of these animals have been described and recorded by many observers.[10] However, a study of the arrangement of the musculature of the branchial area among ganoids and teleosts makes it evident that, though an operculum is characteristically developed in both groups, yet in the relative specialization of this apparatus as a respiratory organ, the two groups present important differences.

Among most teleosts the operculum acts in conjunction with the maxillary, mandibular and branchiostegal valves as a highly efficient pumping mechanism which maintains the flow of water from the oral cavity outwards over the gills through the opercular cleft. In consequence of this, the intrinsic branchial musculature among teleosts is relatively poorly developed, while the respiratory action of the m. adductor mandibularis (closure of the mouth during expiration) is largely abolished (vide infra).

In ganoids, on the other hand, though rudiments of the maxillary and mandibular valves may be represented (Allis, 2), yet their efficiency as such is negligible, so that during expiration the mouth must be closed to direct the respiratory flow backwards over the gills.

[10] The only observations on the respiratory movements of the gills in ganoids that I have been able to find in the literature are those of Kouliabko (77). This author carried out a series of experiments upon the isolated heads of various fish, among which were specimens of Acipenser ruthenus and another closely related form which is referred to as the 'costeur' and probably was the great Russian sturgeon, Acipenser husio. No exact study of the mechanism of branchial respiration was made, but Kouliabko brought to light the interesting fact that ganoids are more tolerant of CO_2 than are teleosts. The interest of this observation is increased in view of Baglioni's finding that selachians resemble ganoids in this respect (7).

Further, in Acipenser, Polyodon and Amia the expiratory branchial muscles are relatively more highly developed as compared with the expiratory opercular muscles than is the case among teleosts. Also, the m. adductor hyomandibularis (expiratory opercular muscle) in Acipenser, Polyodon, and Amia is less highly differentiated than among teleosts (Vetter, 95; Danforth, 16; McMurrich, 83).

Thus on purely anatomical grounds, it would appear that the respiratory cycle in ganoids must present a curious admixture of selachian and teleostean characters. In inspiration these characters are chiefly teleostean, viz., contraction of the m. sternohyoideus and the opercular dilator muscle, together with the valvular action of the free border of the operculum. In expiration, on the other hand, selachian characters predominate, viz., the action of the relatively well developed branchial levator and adductor muscles in conjunction with that of the adductor hyomandibularis, and the necessarily coordinate action of the m. adductor mandibularis.[11]

Turning now to a consideration of the arrangement of the motor nucleus in the light of these observations, it becomes evident that the caudal extent of the motor V nucleus, together with the rostral position of the motor VII nucleus, brings the two nuclei innervating the opercular (and jaw) musculature into closer relation to one another than is the case among sharks where an operculum is lacking. In this respect these nuclei present teleostean characters.[12]

On the other hand, the selachian arrangement of the motor VII–IX–X nuclei to form the caudal viscero-motor column appears to be directly correlated with the relative importance of the intrinsic branchial muscles among ganoids and with the es-

[11] The closure of the mouth during expiration is much more important in ganoids than in sharks owing to the teleostean arrangement of the branchial lamellae in the former animals.

[12] It is of interest in the present connection to refer to the peculiar arrangement of the motor nuclei in Chimaera (Kappers, 66, fig. 20). In this animal, in which the operculum is also developed, the isolated rostral portion of the motor VII nucleus charted by Kappers bears a relation to the caudal motor V elements in all essentials identical with that obtaining in ganoids.

sentially selachian character of the communis area in these forms. Further, the simplicity of the arrangement of the elements composing the caudal viscero-motor column is in itself an indication of the absence of any great specialization of the derivatives of the hyo-branchial myomeres in ganoids.

Oculomotor and trochlearis nuclei and roots (Nu. et rad. N. III and IV). The small size of the oculomotor and trochlear nuclei and their roots in all ganoids is indicative of the relatively slight functional importance of the eye in these forms as compared with sharks. In discussing the abducens nerve, mention was made of the evidence furnished by the structure of the oculomotor and trochlear nuclei in support of the view that ganoid ancestors were possessed of a more highly developed optic apparatus than the modern representatives of this group. This evidence rests upon the following facts:—Though these nuclei and their roots are both relatively and absolutely smaller than in sharks, yet the oculomotor nucleus presents distinct signs of specialization, so that within it may be distinguished two secondary nuclei such as elsewhere are found among fish only in teleosts. Further, the definite gap which exists between the oculomotor and trochlear nuclei in all ganoids, is a condition which also obtains elsewhere among fishes only in certain teleosts (e.g., Rhombus, Hippoglossus, etc.) in which the visual apparatus is highly developed.

In conclusion, it may again be emphasized that the arrangement of the motor nuclei in Polyodon furnishes additional evidence of the importance of neurobiotactic influences in the determination of nuclear pattern. The predominantly dorsal position occupied by the motor nuclei in this form and in other ganoids is in correspondence with the relative importance of the dorsal reflex pathways (posterior longitudinal bundle, octavo-motor tracts, etc.). In this respect ganoids resemble sharks. The more ventral position of the abducens nucleus in ganoids than in sharks is explained on the hypothesis that the tecto-bulbar paths were functionally more highly developed in the ganoid stock than in the modern representatives of the group. This hypothesis is borne out by the evidence furnished by the

relations obtaining in the oculomotor and trochlear nuclei. The rostral position of the motor VII nucleus, together with the caudal extent of the motor V nucleus, point to the probability that these characters are dependent upon the development of a modified type of opercular respiration and the consequent more close association of the motor nuclei upon whose coordinate action certain respiratory movements depend.

Finally, it would appear that further evidence for the grouping of modern ganoids together and apart from teleosts is furnished by the close correspondence of the motor nuclear pattern in all ganoids examined and by the characteristic differences of this pattern on the one hand from that of sharks and on the other from that of teleosts.

TELEOSTEI

Motor nuclei in Ameiurus nebulosus and Solea vulgaris

Among teleosts, two forms whose life habits present a very marked contrast, have been selected for study: the flat-fish Solea vulgaris and the siluroid Ameiurus nebulosus.

Occipito-spinal nuclei and roots (Nu. et rad. mot Nn. occ. spin.). In Solea and also in Ameiurus the rostral end of the somatic motor column of the cord is continued for some distance uninterruptedly into the medulla, and there forms the nucleus of origin of certain precervical motor rootlets.

The general term 'spino-occipital' has been made use of in describing this region in selachians and ganoids to distinguish the rostral portion of the somatic motor column of the trunk and its associated motor rootlets. Since the reduction of the spino-occipital elements among teleosts is evidently more extensive than is the case in lower forms (e.g., selachians and ganoids) as well as in many higher forms (e.g., amphibians), it seems desirable to indicate this fact by the use of some term other than spino-occipital in describing this region in teleosts, especially as the arrangement of the elements in the rostral end of the somatic motor column among Teleostei presents certain characteristics distinctive of the group. For this reason in the present de-

scription, Furbringer's term 'occipito-spinal' has been used in its broadest sense.[13]

In teleosts, unlike selachians, the rostral termination of the occipito-spinal motor column, is rounded and blunt, so that in transverse section the distribution of the motor cells in this nucleus closely resembles the arrangement obtaining in the anterior horn of the cord (figs. 26, 29, 40 A and 41 A).
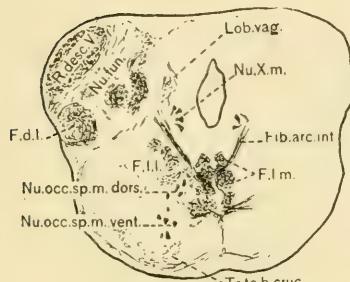
26
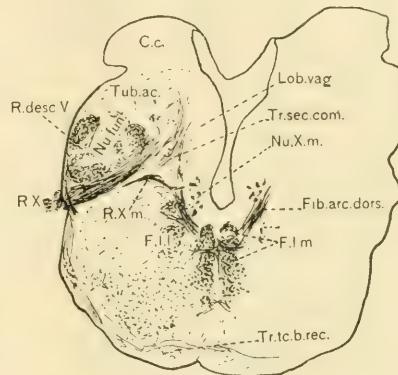
27

Fig. 26   Solea vulgaris.   Transverse section of brain stem just caudad of the calamus.

Fig. 27   Transverse section of brain stem somewhat rostrad of the preceding at the exit level of a motor vagus rootlet.   Abbreviations: *C.c.*, crista cerebellaris; *Fib.arc.dors.*, dorsal arcuate fibers; *Fib.arc.int.*, internal arcuate fibers from funicular area, vagal lobes and motor vagal nuclei; *F.d.l.*, fasciculus dorsolateralis; *F.l.l.*, fasciculus longitudinalis lateralis; *F.l.m.*, fasciculus longitudinalis medialis; *Lob.vag.*, visceral sensory column; *Nu.fun.*, nucleus of funicular area; *Nu.occ.sp.m.dors.*, dorsal moiety of motor occipito-spinal nucleus; *Nu.occ.sp.m.vent.*, ventral moiety of motor occipito-spinal nucleus; *Nu.X.m.*, motor vagal nucleus; *R.desc.V.*, tractus spinalis trigemini; *R.X.*, vagus root; *R.X.m.*, motor vagus rootlet; *Tr.tc.b.cruc.*, tractus tecto-bulbaris cruciatus; *Tr.tc.b.rec.*, tractus tecto-bulbaris rectus; *Tr.sec.com.*, secondary gustatory bundle; *Tub.ac.*, tuberculum acusticum.

[13] In his earlier works Dr. Kappers has used the term spino-occipital to describe this region in teleosts, but the present change in usage has been undertaken on the advice of this author.   It is to be recalled that Furbringer applied the term 'occipito-spinal' to those nerves (viz., a, b, and c) which had only incompletely lost their spinal character and which issued from the cranio-spinal canal rostral to the first true spinal (Furbringer's spinal nerve 4) and caudal to the protometameric portion of the cranium.

In Solea, the fibers of the first motor rootlet which arises from the occipito-spinal nucleus, pass obliquely caudad and ventrad and become collected upon the periphery to form a small bundle which courses some sections caudad in this location before leaving the brain. A similar arrangement obtains in Ameiurus and in other teleosts (cf. Van der Sprenkel, l. c., fig. 4).

The caudal viscero-motor column (Col. visc. mot. caud.). The caudal viscero-motor column in Solea, unlike that of selachians
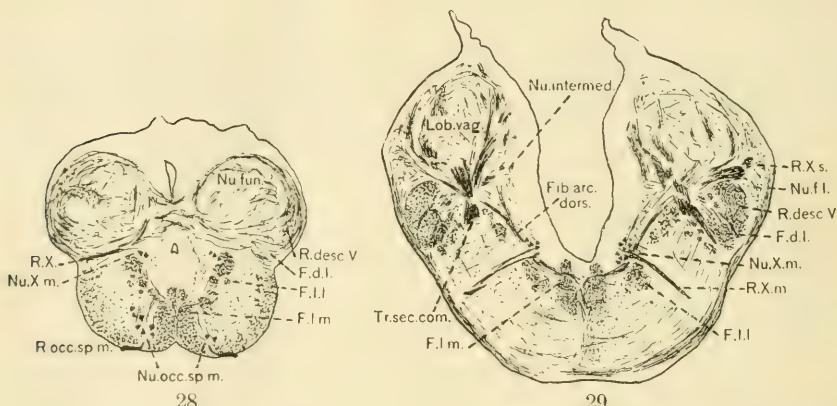
Fig. 28   Ameiurus nebulosus.   Transverse section of brain stem at the exit level of the second occipito-spinal rootlet.

Fig. 29   Transverse section of brain stem somewhat rostrad of the preceding at the exit level of motor vagus rootlets.   Abbreviations: *Nu.f.l.*, lateral funicular nucleus; *Nu.occ.sp.m.*, motor occipito-spinal nucleus; *R.occ.sp.m.*, second motor occipito-spinal rootlet; *R.X.s.*, sensory root bundle of the vagus.   Other abbreviations as in figures 26 and 27.

and ganoids, is divided into two distinct portions:—a rostral part formed by the rostral motor VII nucleus and a long caudal cell column comprising the motor nuclei of the vagus and glossopharyngeus together with the caudal motor VII nucleus. A similar subdivision of the caudal viscero-motor column occurs in Ameiurus, but with this characteristic difference, that the rostral subdivision contains all the cells of origin of the motor facial root, while the caudal subdivision comprises only the motor vagus and glossopharyngeus nuclei.

As in ganoids, the vagus column in teleosts extends caudally a considerable distance beyond the rostral end of the occipito-spinal motor column, and lies dorsal to this nucleus (figs. 26, 29 and 18).

It will be seen from the reconstruction charts in figure 40 that the extent of this overlap may be variable even in closely allied forms. In Solea, the motor X nucleus ends rostrad of the exit level of the first occipito-spinal rootlet, while in Hippoglossus, it extends a considerable distance caudad of the corresponding point. It is to be noted, however, that there is a much smaller amount of variation to be observed when the total length of the VII–IX–X column in these forms is compared. A similar condition is to be seen in a comparison of the reconstruction charts of the siluroids Ameiurus and Silurus, though of course in this case it is the IX–X columns that are to be compared one with the other (figs. 41 A and 42 A).

In Solea, the position of the motor X nucleus in transverse section is illustrated in figures 26 and 27. The motor rootlets of the vagus pass laterally, and emerge ventral to the descending trigeminal root and below their corresponding sensory root. In Ameiurus, the relations of the motor X rootlets and of their nucleus of origin are illustrated in figures 28 and 29.

It is of interest at this point to compare Bartelmez' reconstruction of the motor nuclei in Ameiurus melas (9, fig. 2) with my own chart of these relations in Ameiurus nebulosus (fig. 41 A). The only essential difference in the sagittal relations recorded in the reconstructions, lies in the position of the caudal end of the vagus nucleus with reference to the occipito-spinal motor column. In the specimen of Ameiurus nebulosus from which figure 41 A was prepared, the overlap of the columns in question was considerable; but as no such overlap is indicated by Bartelmez in Ameiurus melas, it is probable that there is a certain amount of 'specific' variation in the extent of this overlap—just as there is a considerable 'generic' variation in this relation among closely allied forms (vide supra Pleuronectidae). If, however, the total length of the IX–X nucleus in A. melas and A. nebulosus be measured and then divided into the distance

between the caudal end of this nucleus and the rostral end of the motor V nucleus, the quotient is expressed by 1.8 in both reconstructions.   Thus, the relative length of the nucleus in question in the two species (Ameiurus nebulosus and Ameiurus melas) is exactly the same.   It is probable that the nucleus which Bartelmez terms "nucleus motorius nervi cervicalis I" corresponds to the nucleus of origin of the first occipito-spinal motor rootlet of my description and not to the motor nucleus of the first true cervical nerve of this form.

Motor glossopharyngeal nucleus and root (*Nu. et rad. mot. N. IX*).   In Solea, the motor glossopharyngeal root, in passing from its nucleus to its point of exit, takes the peculiar roundabout course that appears to be characteristic of this nerve in teleosts.   The motor IX root arises from cells situated in the rostral part of the VII–IX–X column and passes rostrad for a considerable distance alongside the most dorsal bundle of the fasciculus longitudinalis medialis.   At the level of the caudal end of the rostral motor VII nucleus, the fibers of the IX nerve pass through this nucleus laterad and ventrad, but do not receive any fibers from it.   The root then courses caudad and laterad to emerge, ventral to the descending trigeminal tract, at the level indicated in the chart, figure 40 A.   This level is somewhat rostrad of the entrance of the sensory radix of glossopharyngeus.

The course of the motor IX root in Ameiurus closely corresponds to that obtaining in Silurus (Van der Sprenkel, l. c., fig. 4) and differs in no essential way from the course of this root in Solea.   It would appear, however, that among siluroids the 'genu' of the motor IX root, tends to enclose within its concavity a considerable part of the whole motor VII nucleus, while among the pleuronectids, a relatively small part only of the rostral motor VII nucleus lies in this relation.

This peculiar geniculate course of the motor IX root has been described also by Mayser in certain cyprinoids (79) by C. J. Herrick in Menidia (34) and in Hippoglossus, Rhombus, Pleuronectes and Tinca by Kappers, who recently has restudied the

question of the course of the motor glossopharyngeus and its relation to the motor VII nucleus in these forms (72).

Motor facialis nucleus and root (Nu. et rad. mot. N. VII). In Solea, the rostral motor VII nucleus is placed for the most part in the ventricular gray, lateral to the most dorsal fibers of the
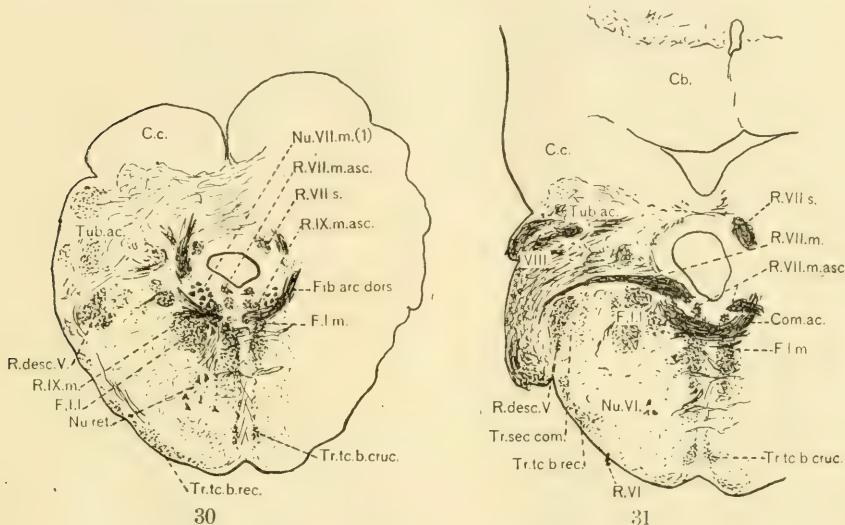
Fig. 30 Solea vulgaris. Transverse section of brain stem to illustrate the relations of the ascending motor glossopharyngeal root.

Fig. 31 Solea vulgaris. Transverse section of brain stem at the exit level of the motor facial root. Abbreviations: *Cb.*, cerebellum; *Com.ac.*, decussating fibers of acusticum; *Nu.ret.*, nucleus reticularis; *Nu.VII.m.(1).*, rostral moiety of motor facial nucleus; *Nu.VI.*, abducens nucleus; *R.VI.*, abducens rootlet; *R.VII.m.*, emergent motor facial root; *R.VII.m.asc.*, ascending motor facial root; *R.VII.s.*, descending sensory facial root; *R.IX.m.*, emergent fibers of motor glossopharyngeus passing laterad through motor facial nucleus; *R.IX.m.asc.*, ascending motor glossopharyngeus root; *VIII.*, lateralis nerve below which the sensory facial root is seen. Other abbreviations as before.

fasciculus longitudinalis medialis, and resting upon the bundles of the fasciculus longitudinalis lateralis (figs. 30 and 40 A). The size of the nucleus increases somewhat towards its caudal end, where many of the motor cells together with their larger dendrites lie in between the bundles of the fasciculus longitudinalis lateralis, and thus occupy a more ventral position than those

at the rostral end of the nucleus. The nucleus is traversed by many thick bundles of the dorsal arcuate fibers.

The motor VII fibers which arise from cells in the rostral end of the VII–IX–X column (caudal motor VII nucleus) pass upwards through the medulla as a small compact bundle, situated just above the most dorsal fibers of the fasciculus longitudinalis medialis (fig. 30). At the level of the rostral motor VII nucleus, fibers from this center join the ascending root. In this way at the level of the upper border of the rostral motor VII nucleus, a large compact bundle is formed which derives its fibers from both rostral and caudal motor VII nuclei. The VII motor root then passes rostrad for some distance and finally curves outwards over the radix descendens V and reaches the lateral surface of the medulla some sections rostrad of the sensory VII root (fig. 31).

In Ameiurus the motor VII nucleus consists of a large mass of cells situated in the tegmentum below the acoustic commissure, resting medially upon the fasciculus longitudinalis lateralis and laterally upon the reticular substance bordering the ascending secondary gustatory tract of Herrick. These relations are brought out in figure 32 and are essentially similar to those obtaining in Siluris glanis. A caudal motor VII nucleus, in continuity with the IX–X motor column, is not present in either Silurus or Ameriurus, so that in these forms all the motor VII fibers arise in a nucleus corresponding to the rostral motor VII nucleus of Solea (fig. 41 A).

The motor VII fibers collect to form a large compact bundle which arches dorsally over the massive acoustic commissure and courses rostrad in the ventricular gray. At the level of the caudal portion of the motor V nucleus, the root curves laterad through the bundles of dorsal arcuate fibers and pierces the secondary gustatory tract to gain the lateral periphery of the bulb (fig. 33).

Abducens nucleus and roots (*Nu. et rad. N. VI*). In Solea, in the ventral tegmentum, at the exit level of the motor VII root, a poorly circumscribed nucleus is found, from which arises the most rostral of the two abducens rootlets (fig. 31). The emer-
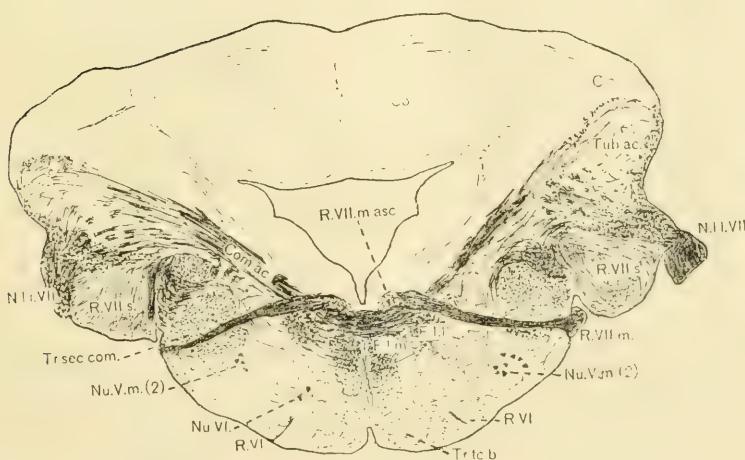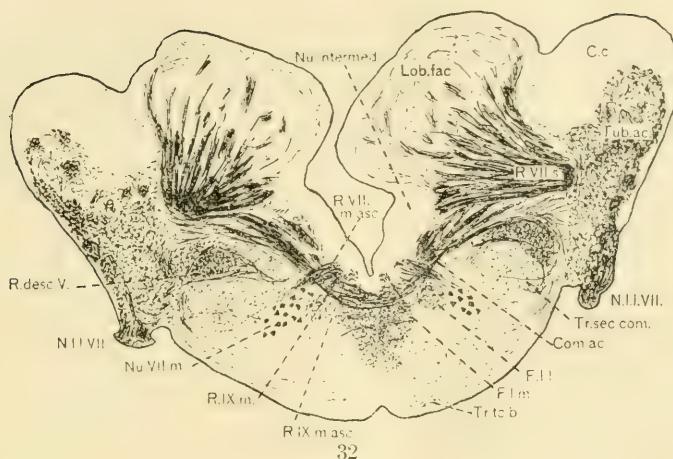
Fig. 32. Ameiurus nebulosus. Transverse section of brain stem at the level of the caudal end of the motor facial nucleus.

Fig. 33 Ameiurus nebulosus. Transverse section of the brain stem at the exit level of the motor facial root and rostral abducens rootlet. Compare with Hexanchus (fig. 13), where an abducens rootlet emerges on the exit level of the motor glossopharyngeus. Abbreviations: *Lob.fac.*, facial lobe of visceral sensory column; *N.l.l.VII.*, nervus lateralis; *Nu.intermed.*, nucleus intermedius; *Nu.V.m.(2).*, caudo-ventral moiety of motor trigeminus nucleus; *Nu.VII.m.*, motor facial nucleus; *R.VII.s.*, entering sensory facial root. Other abbreviations as before.

gent fibers from this nucleus pass ventrad and rostrad and make their exit from the ventro-lateral surface of the medulla on a level with the rostral border of the motor VII root. A second abducens nucleus can be made out caudal to the first, and separated from it by a few cell-free sections. The second abducens rootlet, which arises from the latter nucleus, emerges three sections behind the caudal border of the motor VII root. The presence in this region of numerous diffusely arranged reticular elements, has made it difficult to draw a sharp line of demarcation about the limits of the abducens nuclei, and for this reason they are surrounded by dotted lines in figure 40 A.

The arrangement of the abducens nuclei in Ameiurus is essentially similar to that in Solea, though the caudal abducens root is not separated from the emerging motor IX root by so great a distance in Ameiurus as in the former animal. These relations will be evident on comparing figure 40 A and figure 41 A (also figs. 31 and 33).

It is also of interest to note that the abducens nuclei in Bartelmez' reconstruction of Ameiurus melas (l. c.) bear exactly the same sagittal relations to the motor VII and V nuclei that they do to these structures in Ameiurus nebulosus.

Motor trigeminal nucleus and root (Nu. et rad. mot. N. V). In Solea the motor V root arises from two closely associated cell groups, which from their general relations may be termed respectively dorsal and ventral. The dorsal motor V nucleus occupies a position in the ventricular gray just dorsal to the fasciculus longitudinalis lateralis, and extends in this situation, from a short distance in front of the motor V root, to the level of its caudal border (figs. 35 and 40 A). Numerous large dendrites of the motor cells of this nucleus can be seen passing ventrad between the bundles of the fasciculus longitudinalis lateralis, and occupying a position in the tegmentum analogous to that occupied by the ventral nucleus itself at more caudal levels.

The ventral motor V nucleus begins a few sections caudad of the dorsal nucleus, and extends to within a short distance of the rostral border of the emergent motor VII root. In Solea, unlike Hippoglossus, it is somewhat larger than the dorsal nucleus.

The nucleus intrudes its cells and their large dendrites between the bundles of the fasciculus longitudinalis lateralis for some distance into the tegmentum. They pass in the direction of the substantia reticularis grisea which lies below and medial to the descending trigeminus root, and in the neighborhood of the
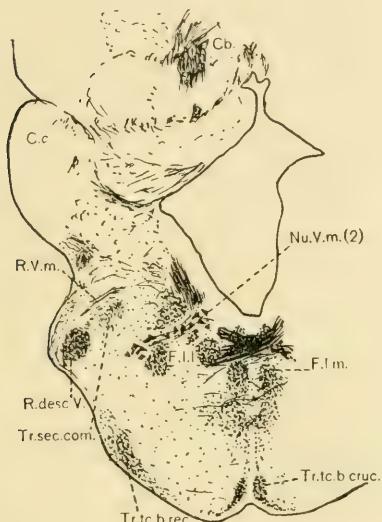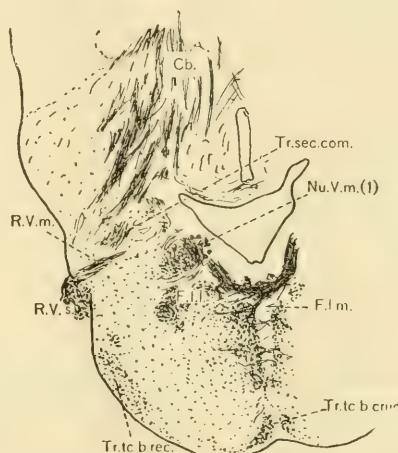
34   35

Fig. 34 Solea vulgaris. Transverse section of brain stem on the level of the caudo-ventral moiety of the motor trigeminus nucleus.

Fig. 35 Solea vulgaris. Transverse section of brain stem on the level of the rostro-dorsal moiety of the motor trigeminus nucleus. Abbreviations: $Nu.V.m.(1)$., rostro-dorsal moiety of motor trigeminus nucleus; $Nu.V.m.(2)$., caudo-ventral moiety of motor trigeminus nucleus; $R.V.m.$, emergent fibers of motor trigeminus root; $R.V.s.$, entering fibers of sensory trigeminus root. Other abbreviations as before.

small secondary ascending gustatory tract (fig. 34). Like the rostral motor VII nucleus, the most caudal cells of the ventral motor V nucleus lie deepest in the tegmentum. The motor V root passes laterad in a curved course and emerges from the medulla dorsal to the sensory root as in Lophius (64).

In Ameiurus, the motor V root emerges in two bundles, as in Silurus, though in the latter form the bundles are not so definitely

separated. The two bundles arise respectively from the rostral and the caudal end of the motor V nucleus. The cells of this nucleus are divided into two closely related groups, so that it is possible to differentiate rostral and caudal motor V nuclei. A similar arrangement of the elements of this nucleus obtains in Ameiurus melas (Bartelmez, l. c.).

Both rostral and caudal motor V nuclei are placed ventrally in the tegmentum. The caudal nucleus extends over the longer
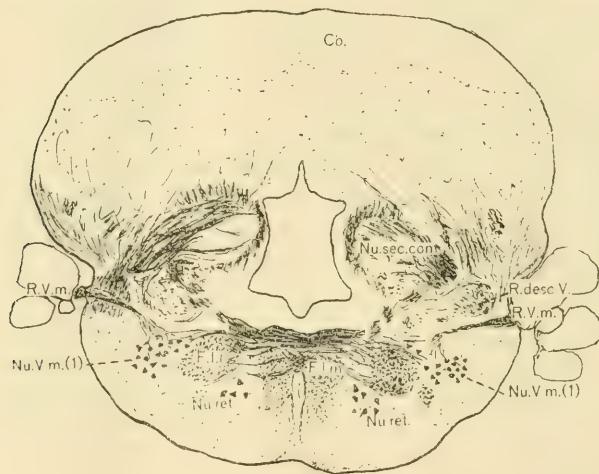
Fig. 36 Ameiurus nebulosus. Transverse section of brain stem on the level of the emergent trigeminus root. Abbreviations: *Nu.sec.com.*, secondary gustatory nucleus; *Nu.V.m.(1).*, rostral moiety of motor trigeminus nucleus; *R.V.m.*, emergent fibers of motor trigeminus root. Other abbreviations as before.

distance sagittally, and is closely related along its dorso-lateral surface to the secondary ascending gustatory tract (fig. 33). The rostral nucleus is somewhat more dorsally placed, and its relations in transverse section are indicated in figure 36. The emerging motor V roots pass almost directly laterad to gain the surface of the medulla, and in their course, they pass through the secondary ascending gustatory tract.

Oculomotor and trochlear nuclei and roots (Nu. et rad. Nn. III et IV). The nuclei of the oculomotor and trochlear nerves are

closely associated in Solea, so that the nucleus of the latter nerve
is separated by but a slight interval from the most dorsal part
of the oculomotor complex. The oculomotor nucleus is situ-
ated in the mesencephalic ventricular gray, and at its rostral
end is made up of a quite compact cell group, lying dorsal to the
fasciculus longitudinalis medialis. As this nucleus is traced
caudad, its dorsal part becomes enlarged, and in addition along
the raphé, an extensive ventro-median cell group appears,
whose elements, though separated into bilaterally symmetrical
groups, give rise apparently to fibers passing to both right and
left oculomotor nerves. The major portion of this nuclear com-
plex lies frontal to the rostral border of the emergent III nerve
but a small part of the ventro-median division of the nucleus
extends a short distance caudad of this level. The emergent
radicles converge in the raphé to form several stout bundles,
which in turn pierce the commissura ansulata in a ventro-lateral
direction to reach the surface of the midbrain (fig. 37).

The trochlear nucleus in Solea, is almost a caudal continuation
of the dorsal part of the oculomotor nucleus, there being but
two cell-free sections separating the two. The IV nucleus is a
compact cell group lying upon the fasciculus longitudinalis
medialis, and extending in this situation approximately over
the length of the superficial attachment of the oculomotor nerve
(fig. 40 A). Thus, in figure 37, it is possible to show the emerg-
ing radicles of N. III, together with the ventral part of nucleus
N. III and nucleus N. IV, all in one transverse section.

The course of the trochlear roots caudad to their points of
emergence is most complicated in Solea. The rootlets become
separated shortly after their origin into two distinct bundles,
one of which after bending around the tractus cerebello-mesen-
cephalicus, decussates with its fellow of the opposite side and
emerges through the valvula. The second rootlet passes rostrad
a short distance in the substance of the valvula, and, after de-
cussating with its fellow at this level, descends between the
valvula and the tectum, to join the rootlet first described at its
point of emergence from the brain.

The conditions which bring about this complicated course of the IV root have been thoroughly investigated, independently by V. Franz (23) and by Huet (l. c.). The latter author has shown that the division of the trochlear root into two bundles and their subsequent decussation at different levels, only occurs among those teleosts having a highly complicated valvula cerebelli
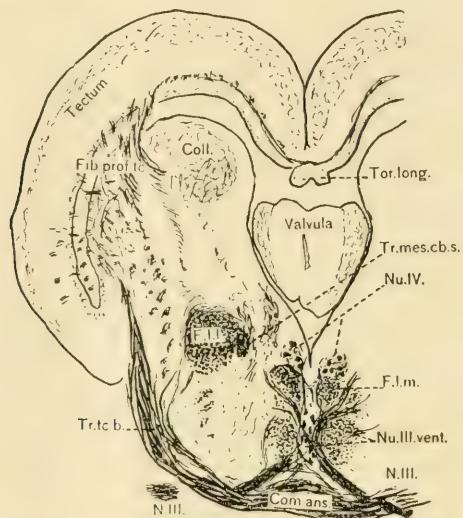
Fig. 37 Solea vulgaris. Transverse section of brain stem on the exit level of the oculomotor root. Abbreviations: *Coll.*, colliculus; *Com.ans.*, commissura ansulata; *Fib.prof.tc.*, fibrae profundae tecti; *N.III.*, oculomotor nerve; *Nu.III.vent.*, ventral moiety of oculomotor nucleus; *Nu.IV.*, nucleus trochlearis; *Tor.long.*, torus longitudinalis; *Tr.mes.cb.s.*, tractus mesencephalo-cerebellaris superior. Other abbreviations as before.

(e.g., Gadidae and Pleuronectidae). In selachians, where no valvula is developed, in ganoids and in teleosts having a relatively small and simple valvula (e.g., Lophius), no such complicated arrangement of trochlear roots obtains. Thus, from the evidence at his disposal, Huet concludes that the action of the valvula cerebelli, in producing this split in the decussating trochlear roots, is purely a mechanical one due to its growth and the consequent folding of the part.

The oculomotor nucleus and root are both relatively and absolutely smaller in Ameiurus than in Solea. It is possible, however, to distinguish dorsal and ventral parts in the oculomotor nucleus of the former animal, though there is but little difference in the rostro-caudal extent of these subsidiary cell groups, such as was so obvious in Solea. No definite decussation of the fibers of the oculomotor nerves at their origin could be made out in Ameiurus, and the whole nucleus lies on a more dorsal plane than in Solea. The emergent roots of the oculomotor nerve pierce the commissura ansulata on their way to the ventral periphery of the midbrain (fig. 39).

The trochlear nucleus in Ameiurus is almost directly continuous with that of the oculomotor nerve but lies on a somewhat more dorsal plane. The emergent trochlear fibers pass dorsally in the Sylvian gray (Franz, l. c.) to decussate and emerge through the valvula on the same level as their nucleus (fig. 38). Though the trochlear roots in Ameiurus have not been divided into two parts as in Solea, yet they make their exit from the brain stem at an unusually rostral level. A similar condition has been recorded by Van der Sprenkel in Silurus glanis, though in this form on account of the very slight functional development of the eyes, both oculomotor and trochlear nuclei were much reduced (fig. 42 A). It would appear that the unusually rostral place of exit of the trochlear roots in both Ameiurus and Silurus, like the splitting of the rootlets in Solea has also been due in a large measure to the mechanical action of the valvula and cerebellum during the development of these parts.

Discussion

Occipito-spinal-complex. In teleosts no pre-hyal hypobranchial spinal muscles are present and according to Furbringer the most rostral spino-occipital nerves represented in these animals are the occipito-spinal roots b and c. In Siluridae according to this investigator both dorsal and ventral roots are represented in the occipito-spinal nerve b, the nerve c is missing, while caudal to and including the first cervical nerve (Furbringer's nerve 4)
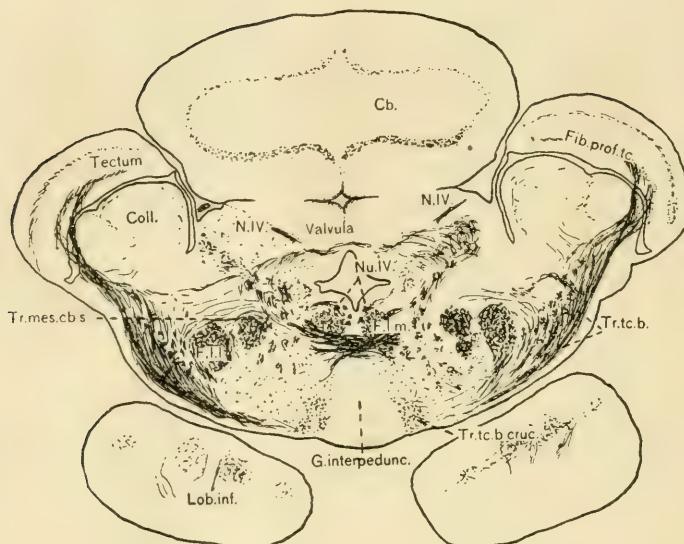
Fig. 38 Ameiurus nebulosus. Transverse section of brain stem on the level of the trochlear nuclei and roots.
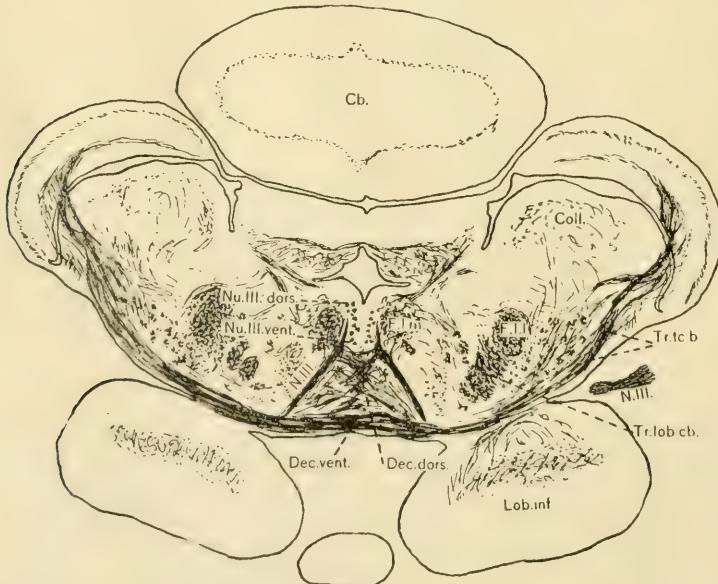
Fig. 39 Ameiurus nebulosus. Transverse section of brain stem on the level of the oculomotor nuclei and roots. Abbreviations: *Dec.dors.*, decussating fibers of fasciculus longitudinalis medialis; *Dec.vent.*, commissura ansulata; *G.interpedunc.*, ganglion interpedunculare; *Lob.inf.*, lobus inferior; *Nu.III.dors.*, dorsal moiety of oculomotor nucleus. Other abbreviations as before.
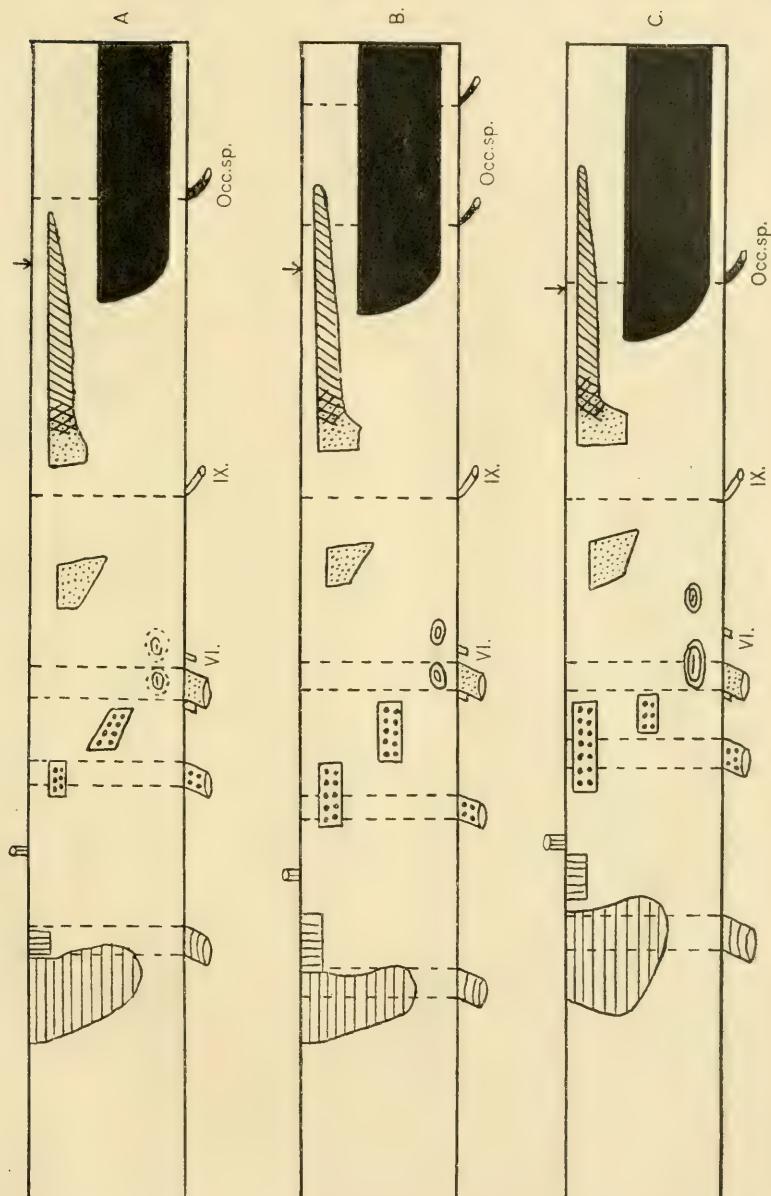
Fig. 40 Reconstruction charts of motor roots and nuclei. A. Solea, B. Pleuronectis (after Kappers, 72), C. Hippoglossus (after Kappers, 72). Signs and abbreviations as before (page 476).
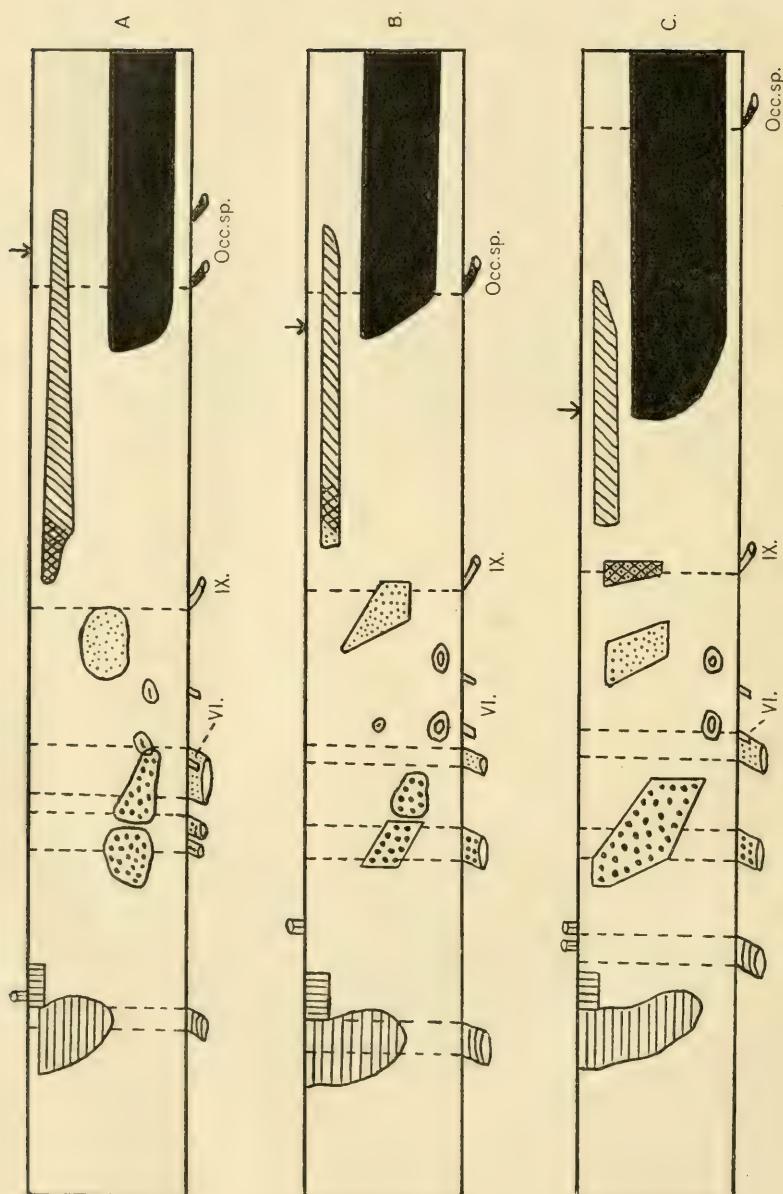
Fig. 41 Reconstruction charts of motor roots and nuclei. A. Ameiurus, B. Tinca (after Kappers, 72), C. Gadus (after Kappers, 72). Signs and abbreviations as before (page 476).
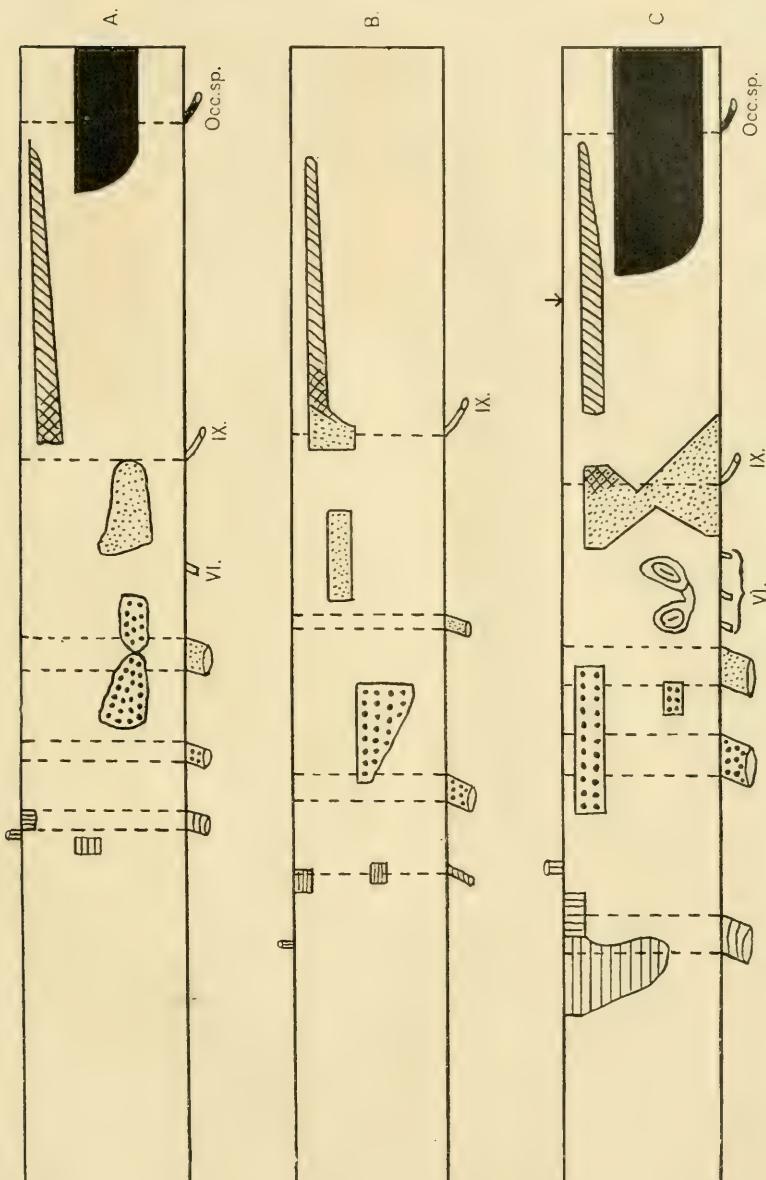
Fig. 42 Reconstruction charts of motor roots and nuclei. A. Silurus (enlarged from Van der Sprenkel, 92), B. Mormyrus (enlarged from Van der Sprenkel, 92), C. Lophius (after Kappers, 72). The charts A and B have been enlarged to the same scale as that used throughout this paper. Signs and abbreviations as before (page 476).

dorsal and ventral roots are regularly represented. In Pleuronectidae the dorsal root of nerve b may be missing and both the dorsal and ventral roots of nerve c are fused with nerve b as they make their exit from the skull but the arrangement of the nerves from this point caudad is similar to that obtaining among siluroids.

The peripheral relations of the cervical nerves in Ameiurus appear to conform closely with Furbringer's generalized scheme for the family outlined above. Thus, according to Wright (l. c.), the first spinal nerve (occipito-spinal b) possesses both dorsal and ventral roots, which unite however, and emerge by a common foramen through the exoccipital bone (auximetameric cranium). The second spinal nerve of this author (Furbringer's nerve 4) is separated from the first by a considerable distance— more than twice the distance intervenes between the exit levels of these two nerves than between those of the second and third. This apparent hiatus in the series would seem to indicate the disappearance of the nerve c. The dorsal and ventral roots of the second nerve emerge through separate dural foramina, as do those of the third nerve, but owing to the modification of the anterior vertebrae in connection with the auditory organ, they pass directly through the membranous wall of the neural canal. The dorsal and ventral roots of the fourth and following cervical nerves emerge through separate foramina in the arches of the corresponding vertebrae.

Within the central nervous system also there are many evidences that both reduction and rostral displacement have occurred in the precervical somatic motor column in Ameiurus as compared with more primitive forms. As these characteristics are typical of all teleosts to a greater or less extent, a general summary of the evidences furnished by the group as a whole will be of value in the present connection.

Evidences of reduction. 1) With the exception of Hippoglossus, in all teleosts examined the distance between the exit level of the first precervical motor root and that of the motor glossopharyngeus is greater than in selachians. 2) Except in the Pleuronectidae, the distance between the exit level of the first

precervical motor root and that of the motor IX is usually greater in teleosts than in ganoids and always greater in teleosts than in certain ganoids (e.g. Lepidosteus). 3) In teleosts a sharp line of demarcation between the motor occipito-spinal nucleus and the coordinating elements of the nucleus motorius tegmenti cannot be drawn. In this respect ganoids stand in an intermediate position between selachians and teleosts. 4) The elements composing the nucleus motorius tegmenti are arranged within the caudal reticular area on a plan almost exactly like that of the rostral occipito-spinal motor nucleus—the only difference between the two lies in the absence of neurones of the peripheral efferent type in the former nucleus, the coordination neurones alone being represented there. 5) The occipito-spinal motor nucleus of teleosts resembles the caudal portion of the spino-occipital nucleus of selachians in the arrangement of its constituent neurones.

Evidence of rostral migration. 1) Though it is known that at least four of the most rostral spino-occipital nerves are not represented in the Pleuronectidae, yet in Hippoglossus the occipito-spinal nerve b is placed as near the exit level of the motor IX nerve as the most rostral occipital nerve is in selachians, (and even nearer than is the case in most of the latter animals). 2) In most Pleuronectidae the distance between the exit level of the motor IX nerve and that of the first precervical motor root is less than in Amia, Acipenser, or Polyodon. 3) The somatic motor nucleus projects further rostrad of its first emergent root in teleosts than is the case in either sharks or ganoids. 4) The most positive evidence of rostral migrations, however, is to be seen among teleosts in the very oblique caudal course taken by the emergent occipito-spinal roots (especially those of the nerve b)—as if the rostral displacement of their peripheral attachments had not kept pace with that of their motor nucleus.

Thus in view of Kapper's earlier work (l. c.) it may be said that, in the absence of manifest mechanical influence, positive evidence of nuclear displacement may safely be adduced when a motor root takes a more or less indirect emergent course, and that the direction of this displacement is indicated by the direc-

tion taken by the emergent fibers. On the other hand, rostral or caudal nuclear migration and emergent root displacement may keep pace so that the direct emergence of a motor root from its nucleus does not altogether exclude the possibility of nuclear displacement.

The presence of both dorsal and ventral cell groups in the occipito-spinal motor column in teleosts in contrast to selachians and ganoids has already been pointed out by Kappers (66 and 74). This author has shown that the more ventral situation of certain of the motor elements of this nucleus in teleosts is possibly one of the direct expressions of the strong reflex influence of the tractus octavo-motorius cruciatus which courses together with the tecto-bulbar fibers along the ventro lateral periphery of the bulb at this level (see also Wallenberg, 96).

In the occipito-spinal nucleus of teleosts the ventral cell group differs in many histological details from the dorsal cells of this nucleus (Kappers, 66). Some light is shed on the different functions of these two cell groups when the peripheral distribution of the first two precervical motor roots in ganoids is compared with that in teleosts.

In Polyodon, where the ventral cell group is lacking in the most rostral portion of the precervical motor column, the first somatic motor nerve of this series does not furnish branches to any lateral (pectoral fin) muscles but is distributed solely to ventral (hypobranchial) spinal musculature (Danforth). The second nerve may also be entirely restricted in its motor distribution to ventral musculature in some individuals (Furbringer). Indeed it is a general rule for ganoids that the first spino-occipital nerve never participates in the formation of the brachial plexus, though the second nerve usually does so. Similarly in selachians the upper members of the motor spino-occipital series take no part in the innervation of pectoral fin musculature (brachial plexus)—and no ventral cell group is present in the rostral end of the spino-occipital column in these forms.

In Ameiurus (McMurrich and Wright, l. c.), however, the first precervical somatic motor root is distributed not only to

ventral musculature (hypopectoralis) but also to lateral trunk musculature (abductors and adductors of pectoral fin). In this animal as in other teleosts a ventral cell group is present in the rostral part and throughout the occipito-spinal nucleus.

In Menidia a similar condition obtains only in addition to motor fibers to the ventral and lateral musculature the first precervical nerve sends out motor fibers to the dorsal trunk muscles of its own segment (Herrick, l. c.).

The relations obtaining in Ameiurus and Menidia may in a broad way be said to hold for all teleosts. At least it is evident that the first precervical motor root in all teleosts becomes distributed to both ventral and lateral musculature, i.e., participates in the formation of both the so-called cervical plexus and the brachial plexus (Furbringer, l. c.).

From this short review it becomes clear that the ventral cell group of the precervical somatic motor column first makes its appearance as a constituent of the most rostral portion of the nucleus in those forms in which the first motor root habitually participates in the formation of both cervical and brachial plexuses. Conversely it is evident that among all forms in which the first precervical somatic motor rootlet is distributed entirely through the branches of the cervical plexus, a ventral cell group is lacking in that part of the nucleus from which the first motor root arises.

It thus emerges that the ventral cell group of the motor occipito-spinal column of teleosts is apparently a nucleus of origin for motor roots distributed through the brachial plexus to dorsal or lateral derivatives of trunk musculature. Further, it would appear that the presence of this ventral cell group in the most rostral part of the precervical somatic motor nucleus in teleosts is due to the phylogenetic loss of the rostral, more specialized, dorsal cell group concerned in other forms in the innervation of epibranchial and pre-hyal hypobranchial spinal musculature, and is probably not the result of a forward migration of ventral elements beneath a phylogenetically older dorsal cell group; for the evidence at our disposal points to the rostral migration of the constituent elements of the occipito-spinal nucleus of teleosts as a whole.

With regard to the cause of this rostral displacement, Kappers
(l. c.) has noted that in teleosts the occipito-spinal nucleus, like
that of the abducens nerve, occupies its most rostral position
among those forms in which the optic reflex systems are most
highly developed (e.g., Pleuronectidae). It would appear prob-
able, however, that the rostral migration of the occipito-spinal
motor complex in teleosts has been determined by influences
quite independent of those acting similarly upon the abducens
nucleus.

In teleosts the general cutaneous components of the trigeminus
and vagus nerves pass caudad in the radix descendens trigemini
to their chief nucleus of termination in the immediate vicinity
of the funicular nuclei (Herrick, 38, 40 and 41). Though in
Ameiurus some of the trigeminal fibers end in relation to the
deeper layers of the facial lobe (39), by far the greater number
end in the terminal nucleus in the funicular region. The rostral
portion of the funicular region in all bony fishes becomes a center
of the highest importance for the correlation of the tactile im-
pressions originating in the head and trunk. The long conduc-
tion paths of the dorso-lateral fasciculus arise in this center from
which also emerge numerous ventral arcuate fibers to establish
immediate reflex connection with the subjacent somatic motor
nucleus.

In the discussion of the funicular region in Prionotus (l. c.)
Herrick draws attention to Sherrington's observation: "Broadly
speaking, the degree of reflex spinal intimacy between afferent
and efferent spinal root varies directly as their segmental prox-
imity" (89, p. 158). At the rostral end of the cord, however,
owing to the changes brought about by the phylogenetic re-
duction of the precervical segments, the dominant reflex influ-
ence of the first sensory segmental nerves upon the motor column
has been to a large extent suppressed and in its place is substi-
tuted that of the funiculo-trigeminal area. Thus it would ap-
pear that the position of the rostral end of the occipito-spinal
nucleus is determined primarily by that of the nucleus of the
descending trigeminal root and the associated funicular nucleus.

Motor vagal nucleus (*Nu. mot. N. X*). The position of the rostral end of the motor occipito-spinal nucleus relative to the caudal end of the motor vagus column and to the exit level of the first occipito-spinal rootlet is one which is subject to a considerable amount of variation in teleosts, even among closely related forms (vide supra). Consequently the degree of overlap of the occipito-spinal nucleus and the motor vagus column in teleosts is not an accurate measure of the caudal extent of the latter nucleus. However, to estimate the relative development of the caudal end of the motor vagus nucleus it becomes only necessary to compare the total length of this structure in different forms regarding the rostral end in all cases as a fixed point. The reason for this becomes evident when it is recalled that the musculature receiving its innervation from the first three branchial trunks of the vagus among teleosts is subject to but little variation in its relative development, while on the other hand the pharyngo-clavicularis muscles and especially the trapezius muscle may vary considerably both in their relative development and in the character of their innervation.

A trapezius muscle which is innervated solely by the vagus nerve and whose homology with the muscle of this name among selachians can hardly be doubted, has been demonstrated in the following teleosts, Salmo (Edgeworth, 20), Menidia (Herrick, 34), Silurus (Juge, 56), Lophius (Guitel, 31) and Ameiurus (Herrick, 36). A trapezius muscle is absent or represented by a muscle innervated by spinal nerves, in the following teleosts: Esox, Cyprinus, Perca (Vetter, 95), and Gadus (Herrick, 35). The pharyngo-claviculares muscles are innervated by the vagus in an essentially similar manner in each of the following forms: Menidia, Gadus, and Ameiurus (Herrick) and in Silurus (Juge).

Of these forms, the motor nuclei have been studied and reconstructed in Silurus, Ameiurus, Lophius, and Gadus, so that it is possible to institute comparisons here with a considerable degree of accuracy (vide figs. 41 and 42). In the case of Gadus and Lophius, the motor vagus nucleus is completely isolated from the other constituents of the caudal viscero-motor column and it becomes at once evident that the length of the nucleus

in question is considerably greater in Lophius, where the trape-
zius is present, than in Gadus, where the representative of this
muscle is innervated by spinal nerves. The motor vagus nucleus
in both Silurus and Ameiurus is continuous with that of the
glossopharyngeus, but the line of demarcation between these
two nuclei has been determined as the level beyond which no
motor IX fibers can be traced. The total length of the motor
vagus nucleus determined in this way is approximately equal in
the two forms and is evidently greater than that of the isolated
nucleus in Lophius.

From this it would appear that the caudal end of the vagus
nucleus in Silurus, Ameiurus, and Lophius, represents a true
nucleus accessorius, homologous in all respects with that of
selachians, while in Gadus this nucleus is wanting.

In view of the fact that the trapezius muscle of higher forms is
habitually innervated from two distinct sources, it seems highly
significant that a muscle is present in certain teleosts (e.g.,
Gadus) which by reason of its connection with the cranium and
shoulder girdle must functionate as a trapezius, even though its
innervation by spinal nerves must preclude its homology with
the trapezius of selachians.

In the case of Gadus Herrick (35, p. 298) notes: "The muscle
running from the cranium to the pectoral girdle in Gadus is
innervated from the spinals and not from the vagus. It is
therefore, merely a detached portion of the general dorsal mus-
culature." If, however, this muscle in Gadus be considered in
comparison with conditions obtaining in higher instead of lower
forms, the peculiarity of its innervation would not exclude it
entirely from homology with the cucularis.

Evidence that the cucularis of higher forms has been derived
in phylogeny from two different sources is furnished by its double
nerve supply. As the presence of definite somatic components
inextricably mixed within this muscle complex seems limited to
higher vertebrates (Sauropsida and Mammalia), this pecu-
liar condition of fusion must be a comparatively recent phylo-
genetic acquisition. If this be so, both components may be

looked for among lower vertebrates as muscles distinct from one another, though closely associated and synergic in their action.

Among elasmobranchs the well developed trapezius is wholly a specialized visceral muscle, but it is closely associated in its action with somatic muscles innervated by cervical motor nerves. It would thus appear that in these animals a prostadium of the cucularis complex might be recognized in these two distinct though synergic sets of muscles.

In teleosts, however, the elements of the trapezius musculature have necessarily been much reduced owing to the development of the bony operculum. It is probably on this account that both components of the cucularis complex of higher forms are apparently never represented in one individual among bony fishes, but either the visceral element (e.g., Ameiurus, Lophius) or the somatic element (e.g., Gadus) is alone retained. In either case, however, this levator musculature of the shoulder girdle in these forms would appear to be homologous with one or other of the components of the cucularis complex of mammals.

This concept accords well with the facts of embryology and is in harmony with the further phylogenetic history of the accessory nucleus, as well as with its ontogenetic history in mammals.

Motor facialis and glossopharyngeal nuclei and roots (Nu. et rad. mot. Nn. VII and IX). In contrast to the condition obtaining in ganoids and selachians, among all teleosts thus far examined a large portion of the VII motor nucleus is separated from the caudal viscero-motor column and lies more ventrally than the latter in the tegmentum. This sequestration of motor facial elements may affect the whole nucleus (e.g., Silurus, Ameiurus, Lophius), or only its rostral portion (e.g., Tinca, Pleuronectidae).

The relations of the motor IX nucleus to the other constituents of the more primitive caudal viscero-motor column are also highly variable among the different species of teleosts. Thus, the motor IX nucleus together with the elements of the caudal motor VII nucleus may be in direct continuity with the motor X nucleus (e.g., Tinca, Pleuronectidae), or the motor IX nucleus alone may form the rostral end of the motor vagus column (e.g., Silurus, Ameiurus). Further, the motor IX nucleus may

be situated on the level of its root exit intermingled with the elements of the caudal motor VII nucleus but completely separated from the motor X nucleus (e.g., Gadus), or again it may be intimately associated with the motor VII nucleus and form with the latter one large nuclear complex entirely apart from the motor vagus column (e.g., Lophius).

The possible factors operating to produce the characteristic ventral displacement of the motor VII elements in teleosts have been discussed in detail by Kappers (62, 64, 72 and 73). This author has definitely excluded the possibility of the production of this displacement through purely mechanical influences. In addition he has shown that the ventral sequestration of the motor VII nucleus has undoubtedly been strongly influenced by the anterior gustatory tract among forms in which this pathway is well developed; while among those animals in which the anterior taste tract is relatively small, the ventral optic and tactile systems are most probably chiefly responsible for this displacement.

Kappers has also shown that the characteristic geniculate course of the emergent motor IX root in teleosts has been produced largely through the more or less mechanical traction exercised upon it during the ventral migration of the motor VII elements (72). However, with regard to the migration of the perikaryons of the motor IX nucleus, no such direct mechanical influence can be demonstrated and there can be no doubt that the peculiarly variable relations of this nucleus among teleosts are due to the action of neurobiotactic forces of a nature similar to those which have brought about the ventral displacement of the motor VII nucleus in these forms.

In teleosts the arrangement of the musculature innervated by the nerves V, VII, and IX has been strongly influenced in its development by the perfection among these forms of two characteristic organ complexes, viz., (1) the respiratory opercular mechanism, and (2) the pharyngeal tooth-bearing apparatus. The important effect of the development of the opercular type of respiration in ganoids upon the motor V and VII nuclear pattern has already been alluded to and will be discussed again

subsequently. The influence exercised upon the feeding reflexes, and consequently upon the pattern of the motor nuclei involved, by the development of a tooth-bearing apparatus in the pharynx must also be great among teleosts. Especially must this be so among those forms in which this structure becomes functionally of much more importance than the buccal teeth.

In Ameiurus the mm. levatores branchiales from the fourth to the seventh are innervated by branchial branches of the vagus and become inserted into the superior pharyngeal bone to which they "impart a rocking motion . . . . which must be very effective in grinding the food against the inferior pharyngeal" (McMurrich, 82). The nicety of the reflex adjustment of the pharyngeal 'masticatory' apparatus in this animal has been aptly described by Macallum in part as follows: "When the epipharyngeal pads are touched . . . . the pads are thrust down, and at the same time those of the floor are elevated in opposition. This is for the purpose of comminuting the food as it passes into the oesophagus, mere contact of food or other matter serving to bring the pads into action" (80, p. 388).

In Gadus (39) the muscles moving the pharyngeal bones appear to be arranged on quite another and more simple plan, and they are also differently innervated. From Herrick's description I gather that the condition in Gadus closely resembles that in Menidia, in which the pharyngo-branchial bones are moved by but two muscles, viz., the first and second internal levators of the branchial arches. The first levator, which is the smaller, is innervated by the IX nerve and acts both as a levator and a protractor, while the large second muscle receives its nerve supply from the vagus and serves simply as a protractor.

Such variation in the relative complexity of this musculature, as well as its nerve supply, is to be expected in view of the fact that the tooth bearing plates in question are not developed in connection with definite branchial arches but only become attached to them secondarily (McMurrich, 81).

From such scant data as the above it is only possible to point out that in Ameiurus and in Gadus when different muscle com-

plexes have been elaborated to produce movement of the pharyn-
geal plates, a most striking difference in the arrangement of the
caudal viscero-motor nuclei (especially the glossopharyngeus)
is to be observed. It is significant, also, that the motor IX
nucleus is subject to such wide variations in its relations (and
consequently in its reflex connections) among teleosts, while in
no other group of the vertebrate series does this nucleus display
such a lack of conformity to type pattern.

Abducens nucleus and roots (Nu. et rad. N. VI). Among
teleosts the abducens nucleus is evidently composed of two more
or less separated subnuclei which are situated ventrally in the
tegmentum and from each of which emerges a single rootlet.
The more rostral rootlet usually emerges on the exit level of the
motor VII root or a very short distance caudad of this structure
though among the Pleuronectidae it characteristically emerges
rostrad of the motor VII root. The more caudal rootlet usually
emerges at a level which is relatively more rostrad than that
of the frontal abducens rootlet of selachians (figs. 17 and 41).
In exceptional cases the abducens nerve may arise from the
brain stem from three rootlets as in Lophius.

The most rostrad of the abducens sub-nuclei lies either on
the level of the emergent motor VII root or but a short distance
caudal of this structure and the second sub-nucleus is situated
slightly caudad of the exit level of the second abducens rootlet.
In Tinca Kappers has described four abducens sub-nuclei,
three of which are represented in his chart of the motor nuclei
(fig. 41 B), while the fourth part, which lies mediad of the ros-
tral ventral sub-nucleus and on the same level, could not be
represented in such a reconstruction.

The relations of the abducens nucleus and of its emergent root-
lets noted here have already been fully described and discussed
by Kappers (63, 64, 66). This author has shown that the fronto-
ventral position of the abducens nucleus in teleosts as compared
with selachians stands in direct relation with the great develop-
ment of the ventral tecto-bulbar paths in the former animals.
Among flat-fish the relatively enormous development of the
tecto-bulbar tract is to be directly correlated with the fact

that among these forms abducens nuclear elements occupy a more rostral position than obtains in any other group of vertebrates yet investigated.[11]

Motor trigeminus nucleus and root (*Nu. et rad. mot. N. V*).   The motor V nucleus among teleosts is, with few exceptions, divisible into two quite definite sub-nuclei.   Of these, one is rostro-dorsal in position, while the other is more caudo-ventrally placed. Kappers has demonstrated that the chief factor responsible for the ventral position of trigeminal elements in teleosts, in contrast to selachians and ganoids, is the neurobiotactic influence of the secondary ascending gustatory tract (64, 66).   This author has also shown that the caudo-ventral cell group of this nucleus is even more intimately associated with the secondary gustatory tract than are the elements of the dorsal cell group, and is largest and most ventrally placed among those forms in which this tract is best developed (l. c.).

A study of the mechanics of respiration among teleosts brings out the fact that the different muscles innervated by the motor fibers of the trigeminus are by no means equally concerned in the respiratory act.   In view of the direct bearing which such considerations must have upon the question of the arrangement of the reflex nuclear pattern in these forms, it becomes desirable before further discussion to review briefly the recent results of investigations in this field.

The respiratory current in most teleosts is produced chiefly by the action of the opercular apparatus in conjunction with the maxillary, mandibular and branchiostegal valves in a manner to which reference has already been made (Dahlgren, 15; Baglioni, 8).

According to Deganello (18) the principal muscles concerned in these movements are the following.   In *inspiration:* (a) m. sterno-hyoideus (spinal nerves 1 and 2); (b) m. dilator operculae

---

[11] It is worthy of note in view of the small size and the lack of compactness of the abducens nucleus in Pleuronectidae, despite the relatively great importance of the oculomotor apparatus in these forms, that according to Harman (33) the external rectus muscle is peculiarly subject to variation among flat-fish and in most of these forms showed evidence of considerable reduction.

(R. mand. V); (c) m. levator arcus palatini (R. mand. V); (d)
m. levator operculi (R. operc. VII). In *expiration:*[15] (a) m.
adductor operculi (R. operc. VII); (b) m. adductor arcus pala-
tini (R. mand. V); (c) m. adductor hyomandibularis (R. operc.
VII); (d) m. geniohyoideus (R. mand. V.+R. operc. VII).[16]

The action of the opercular apparatus is reinforced to a vari-
able though usually slight extent by the intrinsic branchial
musculature as follows. In *inspiration:* (a) mm. interarcuales
ventrales (both oblique and transverse group) (Rr. branch. IX–
X); mm. interarcuales dorsales (Rr. pharyng. X). In *expira-
tion:* (a) mm. levatores arcum branchialium externi et interni
(Rr. branch. IX–X); (b) m. hyohyoideus (R. operc. VII).

In addition to the opercular and branchial respiratory mechan-
ism, the branchiostegal apparatus may play a most important
rôle in the production of the respiratory current in certain marine
forms (e.g., Lophius). Borcea (11) has pointed out that the
development of the branchiostegal apparatus and its muscula-
ture (m. hyohyoideus and special adductors and abductors)
occurs in inverse ratio to the development of the opercular
mechanism. Baglioni (8), carrying this investigation further,
has arranged teleosts into groups depending upon the type of
their respiratory mechanism. Of these groups it will only be
necessary to mention three in the present connection, viz.,
(1) those in which the branchiostegal apparatus is practically
wanting (e.g., Conger); (2) those in which both branchiostegal
and opercular mechanisms are more or less equally well repre-
sented (e.g., Pleuronectidae); and (3) those in which practically

---

[15] The m. geniohyoideus, whose action was considered to be inspiratory by
Deganello and others, has been shown by Holmquist (46) to be chiefly an ex-
piratory muscle. On account of its action, as well as for other reasons, the
muscle has been termed by this investigator the m. protractor hyoidei.

[16] The extent to which the fibers of the facial nerve contribute to the innerva-
tion of this muscle is subject to some question. In Ameiurus few motor VII
fibers can reach the geniohyoideus owing to the very slight anastomosis between
the mandibular V and opercular VII branches (Herrick, 36). The anastomosis
between these nerves seems to be more extensive, however, in Gadus (35) where
the condition resembles that obtaining in Amia (1), but in Menidia (34) the m.
geniohyoideus is supplied wholly by motor trigeminal branches.

the whole respiratory current is maintained through the action of the branchiostegal apparatus (e.g., Lophius).

This review makes it evident that among teleosts the m. adductor mandibulae does not play any important part in respiration. Indeed, it is concerned almost wholly either in adduction or protraction of the lower jaw or in producing traction upon the maxilla.

The musculature innervated by the elements of the motor V nucleus is thus divisible into two quite distinct functional complexes: one whose action is necessarily of a rhythmic character and intimately associated with that of the opercular muscles innervated by the motor VII; and another complex, which is concerned almost wholly with movements necessary for the primary ingestion of food.

The importance of gustatory stimuli in the reflex activity of the respiratory musculature has already been pointed by Kappers. In many forms, however, the gustatory sense plays but little, if any part in initiating the reflex action of the jaw musculature (e.g., Lophius and the Pleuronectidae) and in such forms the influence of visual impressions upon this reflex is great.

The subdivision of the motor V nucleus in most teleosts into two groups has already been described but the significance of the arrangement becomes increasingly evident when correlated with the peripheral conditions outlined above. Thus in Lophius, in which respiration is carried on chiefly through the action of the branchiostegal apparatus while the opercular musculature is reduced to a minimum, the motor VII nucleus is strikingly specialized both as to size and position, while the component groups of the motor V nucleus are also peculiarly modified. The small ventral nucleus in relation to the slightly developed secondary gustatory tract evidently may be correlated with the slight development of the effector organ with which it appears to be associated, viz., the trigeminus opercular musculature. The large dorsal cell group, on the other hand, lies in close relation to the tecto-bulbar tracts in the laqueus, and in size and

importance corresponds to that of the jaw musculature which in this form is exceedingly well developed.

A comparison of the reconstruction charts brings out the additional fact that the independence of the rostro-dorsal motor V nucleus from the caudo-ventral moiety varies directly with the development of the visual apparatus, being greatest in Lophius and the Pleuronectidae and least in Ameiurus and Tinca. Further, this independence of the cell groups of the motor V nucleus varies indirectly with the development of the gustatory apparatus, being greatest in Lophius and the Pleuronectidae, where the secondary ascending gustatory tract is relatively small, and least in Ameiurus and Tinca, in which this system is highly developed. In regard to the functional development of the neurone systems contrasted above, Gadus occupies an intermediate place, a condition which is accurately reflected in the arrangement of the elements of the motor V nucleus in this form.

Though many further examples might be cited of the variations of the moieties of the motor V nucleus in conformity to peripheral development, especially when the relations of this nucleus are compared to this end among the Pleuronectidae, sufficient has been said to indicate that the dorsal group of motor trigeminal elements is most probably concerned in the innervation of the musculature of the jaw, while the ventral group functionates chiefly in the supply of the trigeminal opercular muscles.

Oculomotor and trochlear nuclei and roots (Nu. et rad. Nn. III et IV). The peculiar and complex relations of the emergent trochlear root fibers have already been discussed in connection with the description of this nerve in Solea. However, the position, make up and mutual relations of the oculomotor and trochlear nuclei among teleosts present certain points of interest and will require some further consideration here.

Kappers has already pointed out that the oculomotor nucleus as a general rule occupies a more rostral position among teleosts than among selachians and broadly speaking this teleostean characteristic may be correlated with the increased relative importance of the tectum opticum and its efferent pathways among

these forms. Certain it is that the dorsal and ventral cell groups of the oculomotor nucleus are definitely placed in relation respectively to the dorsal and ventral decussations of the tecto-bulbar tracts (66).

The differentiation of the elements of the oculomotor nucleus into dorsal and ventral moieties reaches its maximum development in fish, among members of the teleostean group. The probable significance of the presence of dorsal and ventral oculomotor cell groups among ganoids has already been alluded to and it remains only to point out that this differentiation, which apparently must have been already forecast if not completed in the ancestral teleostome stock, would seem to be correlated rather with specialization in intrinsic effectors than in the extrinsic oculomotor apparatus. The reason for such a supposition becomes evident when it is recalled that, among modern fishes, in the general arrangement of the extrinsic oculomotor apparatus there are no characters of a truly fundamental nature distinguishing teleostomes from selachians (Harman, 33; Herrick, 34; Workman, 98).

In the unusually small size of its trochlear nucleus Solea presents a marked contrast to the condition obtaining among other members of the flat-fish group in which, as in Lophius, this nucleus is well developed. In this connection the observations of Harman (1. c.) are of further interest. This investigator has pointed out that among flat-fishes the superior oblique muscle is of unusually large size relative to the other eye muscles and that in general, in fish when the visual axes are capable of convergence, there occurs a specialization of the m. obliquus superior. In view of this, it is possible that further investigation may show that the small size of the trochlear nucleus in the specimen of Solea here studied is merely an individual variation.

CONCLUSION

Among myxinoids the most important information concerning environment must reach the central nervous system through olfactory or tactile channels. The restrictions imposed upon these forms by the absence of a functional visual apparatus

together with the relatively slight development of gustatory and vestibular organs have necessitated a corresponding readjustment of their life habits and a consequent limitation of their range and simplification of their motor reactions. In order to exist at all these animals must seek and occupy a favorable environment where adequate protection may be had and where food may be obtained successfully despite the limitation of their sensory equipment.

Success under these circumstances means specialization and the degree of success with which myxinoids have attained this end is admirably exemplified both by the survival of the type under modern conditions and by the highly specialized motor nuclear pattern already described.

That the motor nuclear pattern is specialized in myxinoids is but another way of stating that the neurones in question occupy a position most favourable for the reception of reflex impulses from the dominant afferent nuclei of these animals.

Among elasmobranchs the vestibular, visual and lateral line sense organs are all functionally well developed and with the aid of gustatory and tactile sensibility provide ample means whereby these forms may receive information of a most varied character concerning their environment. Within the brain stem in these forms the terminal sensory nuclei are developed in correspondence with this receptor equipment so that, in contrast to myxinoids, no one constituent of either somatic or visceral areas can be said to dominate the anatomical arrangement of the medulla. On this account the organization of the afferent divisions of the nervous system within the medulla is of a more generalized type among elasmobranchs than in myxinoids.

One of the chief differences between the motor nuclear pattern of cyclostomes and that of elasmobranchs lies in the association of the motor VII elements with those of the vagus and glossopharyngeus to form the caudal viscero-motor column among all members of the latter group. This arrangement has undoubtedly taken place under the influence of the elements within the well developed communis area and has resulted in

placing the motor nuclei of the nerves to the hyobranchial musculature in intimate association with the chief afferent center acting upon them reflexly, in conformity with the first concept of neurobiotaxis.

The most striking characteristic of the motor nuclear pattern of elasmobranchs is its relative fixity in all members of the group. Thus, as regards their nuclear pattern, the most primitive shark resembles the most specialized ray much more closely than such allied teleostean forms as Tinca and Gadus resemble one another.

However, that this reflex organization within the elasmobranch brain stem has been entirely adequate for the needs of these animals is indicated by the successful manner in which the members of this group have competed with other more specialized and modern types in practically every variety of marine environment.

The reduction of the visual apparatus among ganoids has apparently been followed by no marked compensatory development of other of the special senses and has been accompanied indeed by a great reduction of the functional development of the cerebellum as compared with sharks. Apart, however, from the relatively small tectum opticum and the reduced cerebellum the general organization of the afferent functional divisions of the ganoid brain stem is on the whole more selachian than teleostean in character.

The selachian arrangement of the communis area among ganoids has necessarily affected the distribution of the elements of the caudal viscero-motor column. Thus, except for the generally more rostral position of the motor facial elements, the viscero-motor nuclear pattern of these animals is essentially similar to that of sharks.

On the other hand, the effector organs of the head region in ganoids are modified in general away from the selachian type, so that especially in the arrangement of the gill laminae and operculum these forms present important teleostean resemblances.

The peculiar combination of central selachian and peripheral telesotean characters noted above is apparently an evidence of

the inability of the central nervous system in ganoids to specialize *pari passu* with changed peripheral conditions. Definite evidence of this loss within the central nervous system of the capacity for unlimited specialization (suppression of neurobiotactic activities) is to be seen in the restricted distribution of modern ganoids.

Among teleosts the capacity for apparently unlimited variations in the reflex pattern of the brain stem nuclei reaches its acme among the vertebrate series. Within this group the extreme specialization of any of the organs of special sense is followed by a corresponding amplification of the primary afferent nucleus or nuclei involved, together with a modification of the motor nuclear pattern in perfect harmony with reflex needs of the animal. Further, the specialization of effectors of whatever nature is also followed by an adequate corresponding adjustment of the reflex connections of the sensory and motor neurones involved.

Thus, within the teleost group it is difficult to construct any generalized scheme of motor nuclear arrangement owing to the characteristic ability of the elements of the primary nuclei to react to neurobiotactic influences and to develop a pattern based chiefly upon peculiar specific requirements.

LITERATURE CITED

1 Allis, E. P. Jr. 1897 The cranial muscles and cranial and first spinal nerves in Amia calva. Jour. Morph., vol. 12, pp. 487–808.

2   1900 The premaxillary and maxillary bones and the maxillary and mandibular breathing valves of Polypterus bichir. Anat. Anz., Bd. 18, pp. 257–289.

3 Ayers, H. and Worthington, J. 1907 The skin end organs of the trigeminus and lateralis nerves of Bdellostoma dombeyi. Am. Jour. Anat., vol. 7, no. 2, pp. 327–336.

4   1908 The finer anatomy of the brain of Bdellostoma dombeyi. I. Acustico-lateral system. Am. Jour. Anat., vol. 8, no. 1, pp. 1–16.

5   1911 The finer anatomy of Bdellostoma dombeyi. II. The fasciculus communis system. Jour. Comp. Neur., vol. 21, no. 6, pp. 593–617.

6 Babák, E. 1913 Die Mechanik und Innervation der Atmung. Handb. d. vergleich. Physiol. herausg. v. H. Winterstein. Jena, Bd. 1, 2nd Hälfte, Sect. XIV: Fische, pp. 597–706.

7 Baglioni, S. 1907 Der Atmungsmechanismus der Fische. Zeitschr. f. allg. Physiol., Bd. 7, pp. 177–182.

8   1910 Zur vergleichenden Physiologie der Atembewegungen der Wirbeltiere. Teil I: Fische, Ergeb. d. Physiol., Bd. 9, pp. 90–137.

9 Bartelmez, George W. 1915 Mauthner's cell and the nucleus motorius tegmenti. Jour. Comp. Neur., vol. 25, no. 1, pp. 87–128.

10 Bergmann, C. and Leuckart, R. 1852 Vergleichende Anatomie und Physiologie. Stuttgart.

11 Borcea, J. 1907 Observations sur la musculature branchiostégale des Téléostéens. Ann. Sc. de l'Univ. de Jassy T. 4, Fasc. 3 and 4, (cited from Baglioni, 7).

12 Bridge, T. W. 1904 Fishes. Cambridge Nat. Hist. Series, vol. 7, Macmillan.

13 Collinge, W. E. 1894 The sensory canal system of fishes. Quart. Jour. Micr. Sc., vol. 36, pp. 499–537.

14 Couvreur, E. 1902 Sur la méchanisme respiratoire de la torpille. Comp. rend. Soc. Biol., T. 54, p. 1252 (cited from Babák, 6).

15 Dahlgren, U. 1898 The maxillary and mandibular breathing valves of teleost fishes. Zool. Bull., vol. 2, Boston, pp. 117–124.

16 Danforth, C. H. 1913 The myology of Polyodon. Jour. Morph., vol. 24, no. 1, pp. 107–146.

17 Darbishire, A. D. 1907 On the direction of the aqueous current in the spiracle of the dogfish; together with some observations on the respiratory mechanism in other elasmobranch fishes. Jour. Linn. Soc. Zool., London, vol. 30, no. 196, pp. 86–94.

18 Dagenello, U. 1908 Die peripherischen nervösen Apparate des Atemrhythmus. Eine anatomische und experimentelle Untersuchung. Pflüger's Arch. f. d. ges. Physiol., Bd. 123, pp. 40–94.

19 De Lange, S. J. 1910 The descending tracts of the corpora quadrigemina. Folia Neurobiolog., Bd. 3, no. 7, pp. 633–657.

20 EDGEWORTH, F. H. 1911 On the morphology of the cranial muscles in some vertebrates. Quart. Jour. Micr. Sc., vol. 56, N. S., no. 222, pp. 167–316.

21 ELLIOT D. G. 1913 A review of the primates. Monograph I, Amer. Mus. Nat. Hist., New York, vol. 3, Anthropoidia (Miopithecus to Pan).

22 FORTUYN, A. B. DROOGLEEVER 1912 Notiz über den Eintritt der motorischen Nervenwurzeln in die Medulla oblongata und über die Lage der motorischen Kerne bei Amia calva L. Folia Neurobiolog., Bd. 6, pp. 27–30.

23 FRANZ, VICTOR 1911 Das intracraniale und intracerebrale Verhalten des Nervus trochlearis bei den Knochenfischen. Anat. Anz., Bd. 38, pp. 592–598.

24 FURBRINGER, M. 1895 Ueber die mit dem Visceralskelett verbundenen spinalen Musculen bei Selachiern. Jen. Zeitschr., Bd. 30, pp. 127–135.

25 ———— 1897 Ueber die spino-occipitalen nerven der Selachier und Holocephalon und ihre Vergleichende morphologie. Festschrift f. Gegenbauer, Teil 3, pp. 349–766.

26 GADOW, H. 1909 Amphibia and reptiles. Cambridge Nat. Hist. Series, vol. 8, Macmillan.

27 GARMAN, H. 1896 Some notes on the brain and pineal structures of Polyodon folium. Bull. Illinois State Lab., vol. 4, article IX, pp. 298–309.

28 GEGENBAUER, C. 1894 Zur Phylogenese der Zunge. Morph. Jahrb., Bd. 21, Heft 1, pp. 1–18.

29 ———— 1898 Vergleichende Anatomie der Wirbeltiere mit Berücksichtigung der Wirbellosen. Bd. 1, Leipzig.

30 GORONOWITSCH, N. 1888 Das Gehirn und die Cranialnerven von Acipenser ruthenus. Morph. Jahrb., Bd. 13, Heft 3, pp. 427–474.

31 GUITEL, F. 1891 Recherches sur la ligne latérale de le baudroie (Lophius piscatorius). Arch. Zool. Expér., 2 Ser., IX (cited from Herrick, 34).

32 HALLER, B. 1898 Vom Bau des Wirbelthiergehirns. I Teil. Salmo und Scyllium. Morph. Jahrb., Bd. 26, Heft 3 and 4, pp. 345–641.

33 HARMAN, N. B. 1900 The palpebral and oculomotor apparatus in fishes. Jour. Anat. and Physiol., vol. 34, pp. 1–40.

34 HERRICK, C. JUDSON 1899 The cranial and first spinal nerves of Menidia. A contribution upon the nerve components of the bony fishes. Jour. Comp. Neur., vol. 9, pp. 157–455.

35 ———— 1900 A contribution upon the cranial nerves of the codfish. Jour. Comp. Neur., vol. 10, no. 3, pp. 265–316.

36 ———— 1901 The cranial nerves and cutaneous sense organs of the North American siluroid fishes. Jour. Comp. Neur., vol. 11, no. 3, pp. 177–249.

37 ———— 1905 Central gustatory paths in the brains of bony fishes. Jour. Comp. Neur., vol. 15, pp. 375–456.

38 ———— 1906 On the centers of taste and touch in the medulla oblongata of fishes. Jour. Comp. Neur., vol. 16, no. 6, pp. 403–439.

39 ———— 1907 A study of the vagal lobes and funicular nuclei of the brain of the codfish. Jour. Comp. Neur., vol. 17, no. 1, pp. 67–87.

40 HERRICK, C. JUDSON 1907 The tactile centers in the spinal cord and brain of the sea robin, Prionotus carolinus. Jour. Comp. Neur., vol. 17, no. 4, pp. 307–327.

41 · 1908 On the commissura infima and its nuclei in the brains of fishes. Jour. Comp. Neur., vol. 18, no. 4, pp. 409–431.

42 1915 An introduction to neurology. Philadelphia, Saunders.

43 HERRICK, C. J. AND COGHILL, GEORGE E. 1915 The development of reflex mechanisms in Amblystoma. Jour. Comp. Neur., vol. 25, no. 1, pp. 65–85.

44 HOEVELL, J. J. L. D., BARON VAN 1911 Remarks on the reticular cells of the oblongata in different vertebrates. Proc. kon. Akad. v. Wetenschapp., Amsterdam, Mar. 25, pp. 1047–1065.

45 HOLM, J. F. 1901 The finer anatomy of the nervous system of Myxine glutinosa. Morph. Jahrb., Bd. 29, pp. 364–401.

46 HOLMQUIST, O. 1910 Der Musculus protractor hyoidei (genioglossus ant.) und der Senkungsmechanismus des Unterkiefer bei knochenfischen, zugleich ein Beitrag zur Kenntnis der Atembewegungen. Fysiogr. Sällsk. Handlingar Lund., Bd. 21, N. 6, pp. 1–26 (cited from Babák, 6).

47 HUET, W. G. 1911 Notes on the trochlear and oculomotor nuclei and the trochlear root in the lower vertebrates. Proc. kon. Akad. v. Wetenschapp., Amsterdam, Feb. 25, pp. 897–903.

48 HYDE, IDA H. 1904 Localization of the respiratory center in the skate. Amer. Jour. Physiol., vol. 10, pp. 236–258.

49 JOHNSTON, J. B. 1898 Hindbrain and cranial nerves of Acipenser. Anat. Anz., Bd. 14, nos. 22 and 23, pp. 580–602.

50 1901 The brain of Acipenser. Zool. Jahrb., Abt. f. Anat. u. Ontogen., Bd. 15, pp. 59–260.

51 1902 The brain of Petromyzon fluviatilis. Jour. Comp. Neur., vol. 12, no. 1, pp. 1–106.

52 1906 The nervous system of vertebrates. Philadelphia, Blakiston.

53 1908 A note on the presence or absence of the glossopharyngeal nerve in Myxinoids. Anat. Rec., vol. 2, no. 6, pp. 238–239.

54 1909 The radix mesencephalica trigemini. Jour. Comp. Neur., vol. 19, no. 6, pp. 593–644.

55 JORDAN, D. S. 1904 A manual of the vertebrate animals of the northern United States. 9th Edition, Chicago, McClurg.

56 JUGE, M. 1899 Recherches sur les nerfs cérébraux et la musculature céphalique du Silurus glanis, L. Revue suisse de Zoologie, T. 6, Fasc. 1, pp. 1–171 (cited from Herrick, 36).

57 KAPPERS, C. U. ARIËNS 1906 The structure of the teleostean and selachian brain. Jour. Comp. Neur., vol. 16, no. 1, pp. 1–112.

58 1907 Die phylogenetischen Verlagerungen der motorischen Oblongata-Kerne, ihre Ursache und ihre Bedeutung. Neurolog. Centralb., Nr., 18, pp. 834–840.

59 1908 Weitere Mitteilungen bezuglich der phylogenetischen Verlagerung der motorischen Hirnnervenkerne. Der Bau des autonomen Systems. Folia Neurobiolog., Bd. 1, Nr. 2, pp. 157–172.

60 KAPPERS, C. U. ARIËNS 1908 Weitere Mitteilungen über Neurobiotaxis. Folia Neurobiolog., Bd. 1, Nr. 4, pp. 507–534.

61 ———— 1908 Weitere Mitteilungen über Neurobiotaxis, II. Die phylogenetische Entwickelung des horizontalen Schenkels des Facialiswurzelknies. Folia Neurobiolog., Bd. 2, pp. 255–261.

62 ———— 1909 Weitere Mitteilungen über Neurobiotaxis, III. Ueber den Einfluss der Neurone der Geschmackskerne auf den motorischen Facialis und Glossopharyngeuskern und ihr Verhalten zur Radix descendens Nervi quinti. Folia Neurobiolog., Bd. 3, pp. 259–274.

63 ———— 1910 Weitere Mitteilungen über Neurobiotaxis, IV. The migrations of the abducens nucleus and the concomitating changes of its root fibers. Psychiat. en Neurolog. Bl., Bd. 14, pp. 271–306.

64 ———— 1910 The migrations of the motor cells of the bulbar trigeminus, abducens and facialis in the series of vertebrates and the differences in the course of their root-fibers. Verhand. der. kon. Akad. v. Wetenschapp., Amsterdam, Tweede Sectie, Deel 16, Nr. 4, pp. 3–193.

65 ———— 1911 Weitere Mitteilungen über Neurobiotaxis, VI. The migrations of the motor root-cells of the vagus group and the phylogenetic differentiation of the hypoglossus nucleus from the spino-occipital system. Psychiat. en Neurolog. Bl., Bd. 15, pp. 408–427.

66 ———— 1912 Weitere Mitteilungen über Neurobiotaxis, VII. Die phylogenetische Entwicklung der motorischen Wurzelkerne in Oblongata und Mittelhirn. Folia Neurobiolog., Bd. 6, Sommer-Ergänzungs-Heft, pp. 3–142.

67 ———— 1912 The arrangement of the motor nuclei in the brain of Chimaera monstrosa, compared with other fishes. Proc. kon. Akad. v. Wetenschapp., Amsterdam, April 26, pp. 1176–1180.

68 ———— 1913 La signification des fissures du cerveau en général et leur rapport avec les localisations cérébrales intrinséques dans la region insulaire et dans le lobe frontal. Le Névraxe, vol. 14, pp. 217–247.

69 ———— 1913 Cerebral localization and the significance of sulci. Proc. 17th Internat. Congress Med., London, Sec. I, Anat. and Embryol., pp. 273–392.

70 ———— 1913 Phenomena of neurobiotaxis in the central nervous system. Proc. 17th Internat. Congress Med., London, Sec. I, Anat. and Embryol., pp. 109–122.

71 ———— 1914 Ueber das Rindenproblem und die Tendenz innerer Hirnteile sich durch Oberflachen-Vermehrung statt Volumzunahme zu vergrosseren. Folia Neurobiolog., Bd. 8, pp. 507–531.

72 ———— 1914 Weitere Mitteilungen über Neurobiotaxis, VIII. Ueber den motorischen Glossopharyngeus und Facialis bei niederen Vertebraten. Folia Neurobiolog., Bd. 8, pp. 383–394.

73 ———— 1914 Der Geschmack, peripher und central. Zugleich eine Skizze der phylogenetischen Veranderungen in den sensibelen VII, IX and X Wurzeln. Psychiat. en Neurolog. Bl., Bd. 18, pp. 82–138.

74 KAPPERS, C. U. ARIËNS AND FORTUYN, A. B. D. 1909 Researches concerning the motor nuclei of the nervus facialis and nervus abducens in Lophius piscatorius L. Folia Neurobiolog., Bd. 2, Nr. 7, pp. 689–717.

75 KINGSBURY, B. F. 1897 The structure and morphology of the oblongata in fishes. Jour. Comp. Neur., vol. 7, no. 1, pp. 1–36.

76 KISTLER, H. D. 1906 The primitive pores of Polyodon spathula. Jour. Comp. Neur., vol. 16, no. 4, pp. 294–298.

77 KOULIABKO, A. 1907 Quelques expériences sur la survive prolongée de la tête des poissons (I. Comm.). Arch. Internat. d. Physiol., vol. 4, pp. 436–464.

78 KUPFFER, C. VON 1899 Entwickelungsgeschichte des Kopfes. Ergeb. d. Anat. u. Entw., Bd. 5, pp. 563–618, (N. Hypoglossus, pp. 607–628).

79 MAYSER, P 1881 Vergleichende anatomische Studien über das Gehirn der Knochenfische mit besonderer Beruchsichtigung der Cyprinoiden. Zeitschr. f. wissen. Zool., Bd. 36, pp. 259–364.

80 MACALLUM, A. B. 1884 Alimentary canal, liver, pancreas and air-bladder of Ameiurus catus. Proc. Canadian Institute, N. S., vol. 2, no. 3, pp. 387–417.

81 MCMURRICH, J. P. 1884 The osteology of Ameiurus catus (L) Gill. Proc. Canadian Institute, N. S., vol. 2, no. 3, pp. 270–310.

82 ———— 1884 The myology of Ameiurus catus (L) Gill. Proc. Canadian Institute, N. S., vol. 2, no. 3, pp. 311–351.

83 ———— 1885 The cranial muscles of Amia calva (L), with a consideration of the relations of the postoccipital and hypoglossal nerves in various vertebrate groups. Johns Hopkins Univ. Studies, vol. 3, no. 3, pp. 121–153.

84 NEAL, H. V. 1897 The development of the hypoglossus musculature in Petromyzon and Squalus. Anat. Anz., Bd. 13, pp. 441–463.

85 OWEN, R. 1866 On the anatomy of vertebrates. Vol. 1, p. 413, Longmans.

86 POLLARD, H. B. 1892 On the anatomy and phylogenetic position of Polypterus. Zool. Jahrb., Bd. 5, Heft 3 and 4, pp. 387–428.

87 RÖTHIG, PAUL 1913 Contributions upon Neurobiotaxis (the arrangement of the motor nuclei in Myxine glutinosa, in Cryptobranchus, Necturus, Rana fusca and Bufo). Proc. kon. Akad. v. Wetenschapp., Amsterdan, Sept. 27, vol. 16, pp. 296–305.

88 RÖTHIG, P. AND KAPPERS, C. U. ARIËNS 1914 Further contributions to our knowledge of the brain of Myxine glutinosa. Proc. kon. Akad. v. Wetenschapp., Amsterdam, May 30, vol. 17, pp. 2–12.

89 SHERRINGTON, C. S. 1906 The integrative action of the nervous system. Yale University Press.

90 STOCKARD, C. R. 1906 The development of the mouth and gills of Bdellostoma stouti. Am. Jour. Anat., vol. 5, no. 4, pp. 481–517.

91 THEUNISSEN, F. 1914 The arrangement of the motor roots and nuclei in the brain of Acipenser ruthenus and Lepidosteus osseus. Proc. kon. Akad. v. Wetenschapp., Amsterdam, Apr. 24, pp. 1032–1041.

92 VAN DER SPRENKEL, H. BERKELBACH 1915 The central relations of the cranial nerves in Silurus glanis and Mormyrus caschive. Jour. Comp. Neur., vol. 25, no. 1, pp. 5–63.

93 VAN VALKENBURG, C. T. 1911 On the mesencephalic nucleus and root of the N. trigeminus. Proc. kon. Akad. v. Wetenschapp., Amsterdam, May 27, pp. 25–42.

94 VAN VALKENBURG, C. T. 1911 Zur vergleichenden Anatomie des mesencephalen Trigeminusanteils. Folia Neurobiolog., Bd. 5, Nr. 4, pp. 360–418.

95 VETTER, B. 1878 Untersuchungen zur vergleichenden Anatomie der Kiemen- und Kiefermuskulatur der Fische. Jen. Zeitschr., Bd. 12, pp. 431–550.

96 WALLENBERG, A. 1907 Beitrage zur Kenntnis des Gehirns der Teleostier und Selachier. Anat. Anz., Bd. 31, pp. 369–399.

97 WIEDERSHEIM, R. 1907 Comparative anatomy of vertebrates. 3rd Ed., Macmillan.

98 WORKMAN, I. S. 1900 The ophthalmic and eye-muscle nerves of the catfish (Ameiurus). Jour. Comp. Neur., vol. 10, no. 4, pp. 403–410.

99 WORTHINGTON, JULIA 1905 The descriptive anatomy of the brain and cranial nerves of Bdellostoma dombeyi. Quart. Jour. Micr. Sc., vol. 49, N. S., no. 193, pp. 137–181.

100 1905 Contribution to our knowledge of the myxinoids. Amer. Naturalist, vol. 39, pp. 625–663.

101 WRIGHT, R. RAMSAY 1884 On the nervous system and sense organs of Ameiurus. Proc. Canadian Institute, N. S., vol. 2, no. 3, pp. 352–386.

102 1885 On the hyomandibular clefts and pseudobranchs of Lepidosteus and Amia. Jour. Anat. and Physiol., vol. 19, pp. 476–498.

PRIMARY AND SECONDARY FINDINGS IN A SERIES OF ATTEMPTS TO TRANSPLANT CEREBRAL CORTEX IN THE ALBINO RAT

ELIZABETH HOPKINS DUNN

From the Marine Biological Laboratory, Woods Hole, Mass., and the Anatomical Laboratory of the University of Chicago

EIGHT FIGURES

INTRODUCTION

This paper is to report the findings of a somewhat extended attempt to transplant cerebral cortex. In two instances, at least, the attempt to keep alive the neurons within the tissue seemed to meet with success, although not in the manner desired, since the tissue transferred from one animal to a second animal became adherent in a position such that the extending axons could not grow into adjacent nervous tissue.

The secondary findings have some interest also, and the entire problem has a value because of its bearing on the question of the vitality of nervous tissues. Continuation of the life and growth of nervous tissue in vitro has been accomplished successfully, Harrison, '07, Burrows, '11, Lewis, '12, but the perpetuation of the vitality of nervous tissue transferred from one region of the nervous system to another region has met with greater difficulties.

In the earlier attempts at transplantation it was found that the transplanted mass did not disintegrate entirely and disappear, but that the neurons died, leaving the supporting structures to represent the original transplanted portion. Of the earlier attempts at transplantation those of W. Gilman Thompson, '90, of Saltykow, '05, and of Del Conte, '07, may be cited. In

1909, Ranson reported successful transplantation of the spinal ganglion into the brain. Marinesco and Bethe had earlier transplanted ganglia to a position adjacent to the sciatic nerve. In these cases of successful transplantation the neurons remained alive.

In 1904, while research assistant in the Neurological Laboratory of the University of Chicago in charge of Professor H. H. Donaldson, I began some experiments in the transplantation of cortical cerebral tissue in young albino rats.

MATERIAL SELECTED

The albino rat was selected because of its adaptability as a laboratory animal. In contrast to the adult animals which had earlier been selected for attempted transplantation, a more immature animal was utilized. At the ninth or tenth day from birth the cerebral tissue of the albino rat is not mature, the hairy coat is not sufficiently developed to interfere with operation, and although the young must be left in the mother's care after the operation, they are not given that assiduous care which renders operation on very young animals so difficult. It is possible to return the operated young to the nest without fear of attack upon them from the mother, if the precautions mentioned under the discussion of methods of operation are observed.

The records show that in all forty-six rats were operated. Two of these rats died soon after operation. Nine other brains showed nothing of interest on macroscopical examination. Thirty-five brains were sectioned and studied.

For purposes of tabulation as appears in table 1, the forty-six operations are divided into four series made up of an irregular number of groups. The term group had a definite connotation and marked the number of animals operated at one time. A group usually consisted of the young of one litter, the transplantation being made from one to another young rat of one litter. The brains were thus equally mature at time of operation and the rats of the closest consanguinity. It was hoped that these two points might have weight in the preservation of

TABLE 1

	SEX	BORN	OPERATED	KILLED	AGE IN DAYS	DAYS AFTER OPERATION
Series I						
Group I, Rat 1......	?	12/16/1903	12/28/1903	4/ 5/1904	111	99
Group II, Rat 1......	?	12/23/1903	1/ 4/1904	1/12/1904	20	8
Group III, Rat 1......	♂	1/ 4/1904	1/14/1904	4/ 9/1904	96	86
Rat 2......	♂	1/ 4/1904	1/14/1904	4/ 9/1904	96	86
Group IV, Rat 1......	♀	1/12/1904	1/23/1904	4/ 7/1904	86	75
Rat 2......	?	1/12/1904	1/23/1904	4/ 8/1904	87	76
Rat 3......	♀	1/12/1904	1/23/1904	4/ 9/1904	88	77
Series II						
Group I, Rat 1......	♀	5/30/1904	6/ 9/1904	7/12/1904	43	33
Rat 2......	?	5/30/1904	6/ 9/1904	7/18/1904	49	39
Group II, Rat 1......	♂	6/12/1904	6/22/1904	8/10/1904	57	49
Rat 2......	♀	6/12/1904	6/22/1904	8/10/1904	57	49
Group III, Rat 1......	♂	7/10/1904	7/20/1904	10/26/1904	108	98
Rat 2......	♂	7/10/1904	7/20/1904	10/26/1904	108	98
Series III						
Group I, Rat 1......	♂	4/ 7/1905	4/17/1905			
Rat 2......	♀	4/ 7/1905	4/17/1905			
Rat 3......	♀	4/ 7/1905	4/17/1905			
Group II, Rat 1......	?	4/17/1905	4/27/1905	6/ 7/1905	51	41
Rat 2......	♀	4/17/1905	4/27/1905	6/ 7/1905	51	41
Rat 3......	♂	4/17/1905	4/27/1905	6/ 8/1905	52	42
Group III, Rat 1......	♂	5/10/1905	5/20/1905	9/14/1905	127	117
Rat 2......	♂	5/10/1905	5/20/1905	9/14/1905	127	117
Rat 3......	♂	5/10/1905	5/20/1905	9/14/1905	127	117
Group IV, Rat 1......	?	9/11/1905	9/21/1905	11/ 6/1905	54	44
Rat 2......	?	9/11/1905	9/21/1905	11/11/1905	59	49
Rat 3......	?	9/11/1905	9/21/1905	11/21/1905	69	59
Rat 4......	?	9/11/1905	9/21/1905			
Series IV						
Group I, Rat 1......	?	11/24/1906	12/ 4/1906	2/27/1907	95	85
Rat 2......	?	11/24/1906	12/ 4/1906	3/20/1907	116	106
Group II, Rat 1......	♀	2/24/1907	3/ 5/1907	5/ 3/1907	68	59
Rat 2......	♂	2/24/1907	3/ 5/1907	5/ 9/1907	74	65
Group III, Rat 1......	?	4/14/1907	4/22/1907	4/29/1907	15	7
Rat 2......	♂	4/14/1907	4/22/1907	6/26/1907	73	65
Group IV, Rat 1......	♀	4/17/1907	4/26/1907	7/ 1/1907	75	66
Rat 2......	♀	4/17/1907	4/26/1907	7/ 1/1907	75	66
Rat 3......	♂	4/17/1907	4/26/1907	7/ 1/1907	75	66

TABLE 1—Concluded

	SEX	BORN	OPERATED	KILLED	AGE IN DAYS	DAYS AFTER OPERATION
Group V, Rat 1......	♀	5/14/1907	5/23/1907	10/ 4/1907	143	134
Rat 2......	♀	5/14/1907	5/23/1907	10/ 4/1907	143	134
Rat 3......	♂	5/14/1907	5/23/1907	10/ 4/1907	143	134
Rat 4......	♂	5/14/1907	5/23/1907	10/ 4/1907	143	134
Group VI, Rat 1......	♂	5/23/1907	6/ 1/1907	9/24/1907	125	116
Rat 2......	♂	5/23/1907	6/ 1/1907	11/23/1907	185	176
Rat 3......	♂	5/23/1907	6/ 1/1907	12/28/1907	220	211
Rat 4......	♂	5/23/1907	6/ 1/1907	12/28/1907	220	211
Group VII, Rat 1......	♂	6/16/1907	6/27/1907	11/30/1907	167	156
Rat 2......	♂	6/16/1907	6/27/1907	12/28/1907	195	184
Rat 3......	♂	6/16/1907	6/27/1907	12/28/1907	195	184

vitality in the transplanted material. The consanguinity may have particular value if closely related individuals have a similar metabolism and hence a like chemical constitution of the body tissues.

METHOD OF OPERATING

For the convenience of the operator, the left hemisphere of the brain was chosen for operation in each case. A portion of the cerebral cortex was selected for removal, the loss of which would least interfere with the nutrition of the operated animal The skin was first opened near the median line of the head by an incision carried from the region of the eyes to the nape of the neck, an incision of not excessive length in the young albino rat. The flap on the left side was retracted by pulling on the skin at some little distance from the line of incision. Then with fresh sterile scissors a cartilaginous flap was made in the parietal region of the skull. This was accomplished by a crescent-shaped incision with the attached base just above the ear. This procedure was repeated on a second rat. Then with a thin knife a triangular portion of the thin cortex was removed from the first rat and replaced by a similar portion from the second rat. The incision was usually made in such a way that the

apex of the triangle extended downward. The time consumed in the transfer of the cortex was made as short as possible. In the earlier operations the lateral ventricle was often accidentally opened, although the intention had been to remove and replace a thin superficial portion of cortex only. The apparently successful cases were found to be those in which the ventricle had been opened and a bit of cortex had become adherent to the choroid plexus. During and after the operation the dura was preserved if possible but in rats of ten days the cerebral membranes are very delicate and difficult to differentiate from one another. Sometimes the dura remained conveniently attached to the cartilaginous flap. After transference of the cortical mass the cartilaginous flap was freed, the skin drawn over it and the edges of the skin retained in proximity by a collodion dressing.

Attention to a number of details was found to be advantageous. Complete anaesthesia before and during the operation was found necessary, otherwise the struggles of the animal caused protrusion of the cerebral substance through the incision. Asepsis was secured by using successive sets of sterile instruments. No antiseptic was used other than the ether of the collodion dressing. Maintenance of the body warmth both during and after the operation was essential. This care for the maintenance of the body heat and that for the exclusion of antiseptics were the very helpful suggestions of Prof. C. S. Sherrington, who was a visitor to the laboratory during the early experimental period. Care was exercised not to injure adjacent cerebral structures. Transplanted material was handled rapidly and with a warm knife. An almost insuperable difficulty appeared to be that of retaining the transferred material in the desired place. At ten days of age the cerebral cortex of the albino rat is soft and plastic. The removal of tissue leaves an irregular and almost imperceptible cavity, so that the transferred tissue is displaced almost immediately from the convexity of the cerebral hemisphere. In the later series an attempt was made to permit the formation of a thin blood clot lying over the transferred bit of tissue and extending to the

adjacent parts of the hemisphere. This splint would be, I believe, an important factor in ultimate success.

The operation was done under ether anaesthesia and the animal kept warm, both during the operation and for two or three hours after the operation, until it had thoroughly recovered from the anaesthetic, and the odor of the ether from the anaesthetic and from the collodion dressing had disappeared. Then the young rats were returned to the nest and met with no interference from the mother other than futile attempts to remove the collodion dressing. The quiet and seclusion of the nest during the few days after operation aided convalescence. Young rats are not inclined to stray from the nest until the eyes open, about the fourteenth day of life.

EXAMINATION OF THE MATERIAL

No microscopical studies of the early conditions of the transplanted material were attempted, as the attention was centered on an effort to ascertain whether such transplanted material would later contain mature neurons with medullated axons. The brains of a few rats which died soon after operation gave no suggestion of the survival of the transplanted tissue.

A few of the operated brains especially from the rats of the earlier operations, showed some inflammatory changes, with disintegration of the cerebral substance, about the region of the incision.

The rats upon whom these experiments were carried out gave the appearance of normal rats. No convulsions or paralyses were noted in the operated animals. Control rats were studied during the course of the first operations but, when no sequellæ of the operative procedure were noted, the observation of control rats was abandoned.

The examination of the material removed post-mortem was of two kinds. The first of these was the gross examination at the time of autopsy, when notes were made regarding the condition of the skull, of the meninges, and of the cerebral substance as to the superficial extent of the wound, location of the cicatrix, et cetera. The brains were then removed and fixed in

ten per cent formalin. Some weeks before the brains were to be embedded for cutting, they were mordanted in toto in Müller's Fluid. The blocked material was cut serially either in thirty or forty-five micra sections and stained by the Weigert-Pal method. Alternate sections were counter-stained with Upson's Carmine.

The sections were studied individually under low and high power.

I am indebted for the drawings to Miss Katherine Hill and Mr. A. B. Stredain.

The completion of this study was made possible through the courtesy of Dr. R. R. Bensley and Dr. C. J. Herrick, who granted me an additional amount of free time for the microscopical study of the sections.

The rats used were bred in the laboratory, with the exception of Series IV, Group I, for which I am indebted to Dr. J. B. Watson.

THE FINDINGS FOR CEREBRAL TRANSPLANTATION

The investigation now reported was undertaken for the purpose of determining the possibility of maintaining the life of nerve cells in bits of transplanted cerebral cortex. This continuity of vitality has been found possible and growth has gone on in the neurons transplanted. The neurons which have survived have assumed their morphological relations to other neurons within the transplanted bit. The growth changes within the transplants are very similar to those of normal material of about the same age. Medullation is fully accomplished. The number of medullated fibers is however relatively smaller than in normal material but this is probably due to the absence of such fibers as grow into any cerebral region from other parts of the brain. The growth of the individual neurons has been very considerable. Watson, '03, found that the cortex of the brain of the albino rat is but slightly developed at the tenth day of life, the age at which transplantation was attempted, and that medullation appears much later. The transplanted neurons must therefore have been very immature.

The possible relations of these transplanted neurons with neurons outside of the transplanted portions have not been determined by the results of these experiments. In no brain of the four, with successful transplants, did the transplanted bit so attach itself that fibers could cross the line of attachment to unite functionally with adjacent neuron masses.

The two points of chief importance in successful cerebral transplantation are first, the retention in place of the material transferred, and second, the furnishing to it of an adequate blood supply. Apparently the death of neurons in blocks of transplanted cerebral cortex has been due to some factor which has not affected the vitality of other tissues. The supporting tissues of the cortex have lived and retained the mass form of the transplanted bit. This may suggest the lack of sufficient nourishment for the nervous elements. In my own successful operations, the transplanted portions have remained adherent to the denuded portions of the cortex but have taken some position near the choroid plexus of the lateral ventricle and have apparently received their blood supply from that source. Dr. Ranson permits me to mention that in carrying on some further (unreported) studies in the transplantation of nerve ganglia into the brain he found the most nearly normal conditions in those ganglia which were within or adjacent to the choroid plexus. This may have been due to the more complete anchorage of the material or to a more adequate nourishment, and my own experience would put emphasis on the latter reason. It would seem then that after the mechanical difficulties of securing juxtaposition have been solved, the viability of the transplanted tissues will be secured by guaranteeing sufficient nourishment.

My chief reason for believing these four to be true transplantations of cerebral cortex is the finding in each instance a line of cicatricial tissue about the mass of cortex in question. To follow the enclosing cicatrix it was necessary to study serial sections, and to assure oneself that the tissue mass in question was not partly separated from the remainder of the brain, or a bit which had been twisted out of its original position in the

course of the operation and had retained its vitality because of its ability to draw nourishment from its original blood supply.

To illustrate the conditions found in successful transplants, drawings have been made from sections of the brain of Rat 2 of Series IV, Group IV Figures 1 and 2 are drawn from sections 119–120 of this brain. Figure 2 is a detail from the region
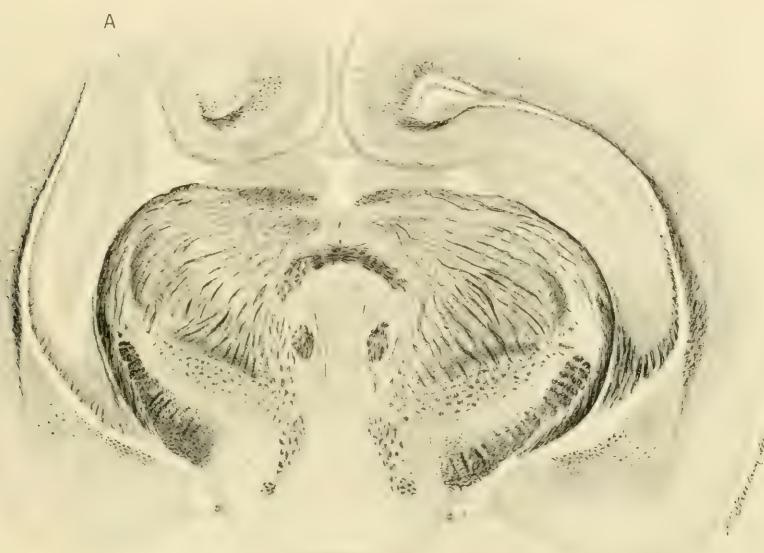
Fig. 1 Showing at *A* a bit of transplanted cerebral cortex in the albino rat. From sections 119–120, Series IV, Group IV, Rat 2. × 7.5.

marked *A* in figure 1. This is by chance the first true transplantation to be noticed as all the material was carried through before detailed studies were made upon the completed slides. Later in the course of reëxamination, other true transplants were observed.

On the discovery of the transplant, figures 1 and 2, it was thought possible that it might be a portion of tissue pinched off from the hippocampus, to which it lies adjacent. However a rather wide band of cicatricial tissue could be seen in the double stained sections, separating the mass from the adjoining

structures. The position of the perikarya, also, and the relations of the medullated nerve fibers are those of a bit of inverted cerebral cortex. The capillary blood supply appears to be derived through the cicatricial adhesion to the plexus choroideus of the lateral ventricle.

Fig. 2 Detail of figure 1. Showing attachment of transplanted portion (*A*) to hippocampus (*H*) at the left. The cortex is inverted and adherent to the choroid plexus (*C. P.*) from which it seems to derive its blood supply.

While the general type of cerebral cortex prevails in the portions transplanted, certain differences from normal cortex can be detected. Such areas have a slightly different reaction to staining agents than have surrounding areas. The colors vary slightly in intensity and in shade from those of the rest of the section. After fixation and staining the neurons appear some-

what fragmented, especially the free endings of the medullated nerve fibers which easily fray. The blood supply is less ample in such regions, the capillaries being more slender and less well filled.

THE MASSING OF CORTICAL FIBERS

In addition to what we may regard as true transplantation of cerebral cortex, other interesting results of the operations were noted. One of these was reported at a joint meeting of the Chicago Neurological Society and the Biological Club of the University of Chicago, March 30, 1909, under the title "On the course of cortical tangential fibers developing after ablation of encephalic cortical substance." Perhaps the use of the term 'tangential' in this connection is misleading. The fibers to which the report refers were parallel to the surface of the brain and located at various depths throughout the cortex. They were not tangential in the narrower sense of the term as it is applied to the fibers lying near the surface of the cortex. At a later time it was noted in other brains that vertical fibers were also apparently increased in number. The materials in which these conditions were noted were produced in the following way. When the operator accidentally opened into the lateral ventricle in the course of operation, there was a tendency for the sub-ventricular substance to protrude through and to widen the original opening. It was while studying serial sections of a brain in which this had occurred that the apparent increase of fibers about the open space was noted. In the normal cerebral cortex of the albino rat many scattered medullated nerve fibers may be found at various depths running parallel to the surface of the cortex. In those brains in which considerable openings occurred, there appeared, in transverse serial sections, to be a massing of fibers parallel to the cortex. These bands of fibers could be traced from outlying cortex and were found to merge into cerebral tissue which had about the normal number of fibers which were parallel to the surface. It seemed at the time that neuron processes which in their growth were not able to follow the path usual to them had been deflected by the wall of the

open space and formed a band along the margin. It appeared
that the massing was more noticeable when the margin of the
opening was near the center of the antero-posterior diameter of
the hemisphere than when it was near the frontal or occipital

Fig. 3 Showing margin of wound with a massing (*M*) of cortical fibers.
These fibers can be traced in successive sections, to the surrounding margin of
the open space produced by the ablation. Zeiss microscope, camera lucida.
Outline on table level with Ocular 2, Objective 16.0 mm.

pole of the hemisphere and that this was correlated with the
marked increase in the number of such fibers in the corre-
sponding region. It seemed possible then to interpret these fibers
as association fibers because they could be found at various

levels in the cortex, could not be traced to projection fibers, and extended some distance through the cortex. Fibers of this kind can be found at M in figures 3 and 4 and in figures 5 and 6.
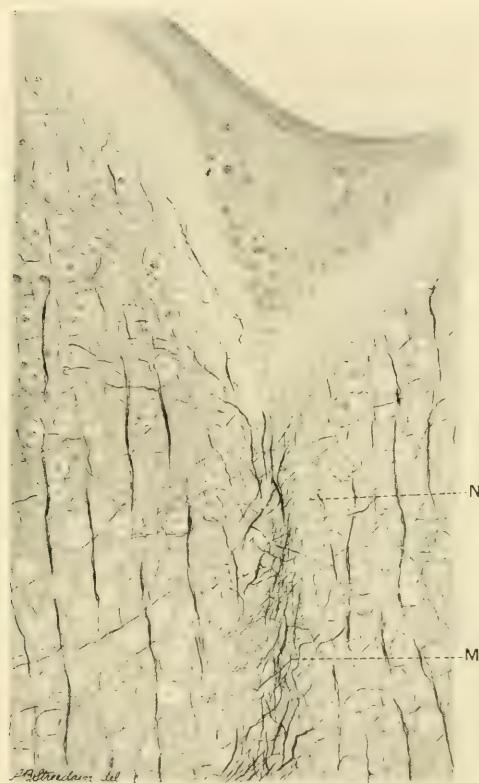
Fig. 4 Detail of figure 3. Showing the upper part of figure 3. Some small neurons (N) may be seen among the massed fibers. Zeiss microscope, camera lucida, outline on table level with Ocular 2, Objective 8.0 mm.

The more recent work of Greenman, '16, suggests a satisfactory interpretation of the number of medullated nerve fibers found circling the gap in the cortex. Dr. Greenman found in studies on the regeneration of peripheral nerves that the number of nerve fibers on the proximal side of the lesion in the regenerating nerve was enormously increased over the normal.

This increase apparently depended on the amount of obstruction offered by the connective tissue through which the nerve fibers must grow to reach their peripheral terminations. This interpretation may well account for the large number of fibers found in such sections as those shown in figures 3 and 4 and figures 5 and 6. The growth of tissue around a gap in central nerve sub-

Fig. 5 Showing massing of cortical fibers along the open wound (*W*). Fibers apparently extend into the internal capsule (*I. C.*). Freehand drawing from Section 121, Series I, Group IV, Rat 3.

stance is comparable to their growth through connective tissue in peripheral nerves and may be governed by the same laws.

THE GROWTH OF MEDULLATED NERVE FIBERS ACROSS CICATRICIAL TISSUE

Ranson, '03, demonstrated that processes of neurons could grow across cicatricial tissue and develop their medullary sheaths. These findings were on stab wounds of the corpus callosum in the albino rat. The cerebral cortex of operated

rats, in my experiments, yielded similar nerve fibers in such instances as the incision had extended for some distance into otherwise normal tissue and the coapted edges had united by the formation of cicatricial tissue. The fibers traversing the cicatricial tissue are no more numerous in my material than they were in that of Dr. Ranson, but are distinctly to be seen and, in favorable sections, may be traced for some distance.
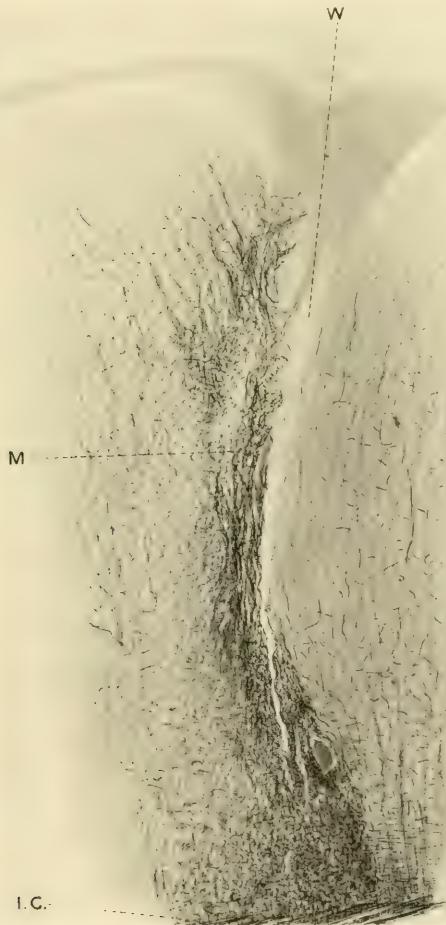
Fig. 6 Detail from figure 5. Freehand drawing. Magnification unknown.

Ranson interpreted these fibers as processes of neurons which were immature at the time of operation. Their perikarya may be located at some distance from the cicatrix. At F, in figures 7 and 8, may be noted such medullated nerve fibers crossing cicatricial tissue in the cerebral cortex of an albino rat.
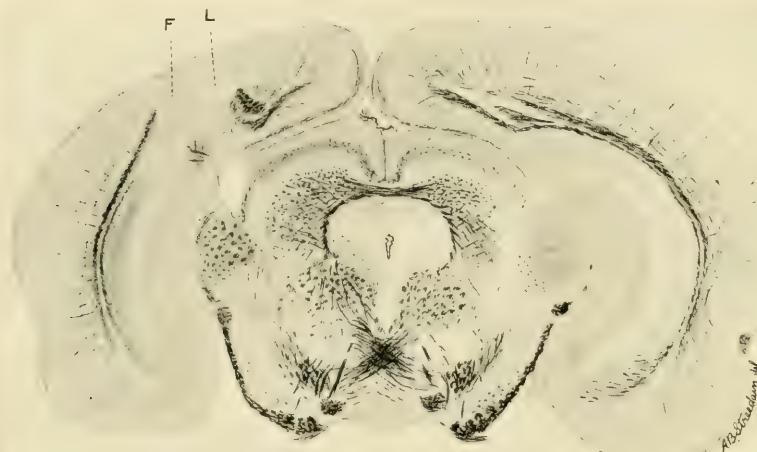
Fig. 7 Showing a section of the brain of the albino rat from a region near the posterior pole. In the dorsal part of the left hemisphere is a light line (L) marking the line of incision, with a few medullated nerve fibers crossing it (F). From sections 81–82, Series IV, Group IV, Rat 3. × 6.

SUMMARY

By the use of immature nervous tissue from the brain of the albino rat the life of the constituent neurons in the cerebral cortex has been maintained after transplantation.

After many unsuccessful attempts this result was obtained by utilizing a thin covering blood clot to retain the graft in position.

The best nourished grafts were those which lay near the plexus choroideus of the lateral ventricle.

In the neurons of the transplanted cortex certain differences from those of normal tissue were detected. These differences were in the staining intensity and morphology of the perikarya and medullated fibers. The blood supply was less ample.

A massing of tangentially placed medullated nerve fibers was found about the open spaces produced by accidental ablations of cortex. These tangential fibers probably connect different parts of the cerebral cortex. This aberrance of nerve fibers shows that a new path may be routed when the usual path has been permanently blocked.
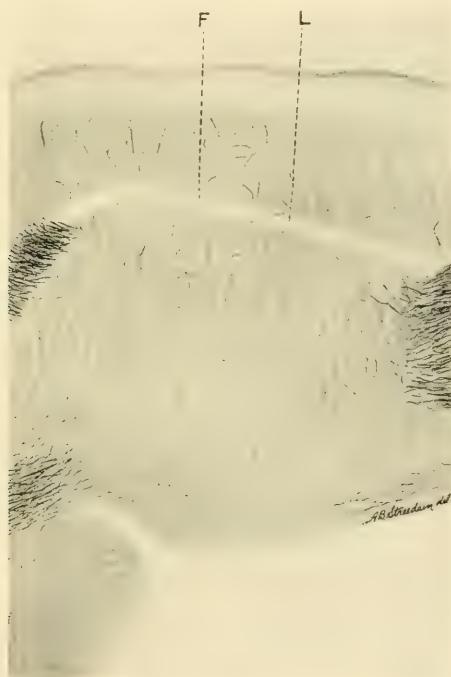
Fig. 8 Detail from figure 7, showing the line (L) of cicatricial tissue with several medullated nerve fibers (F) crossing it. × 28.

Similar bands of projection fibers were noted along the margins of incised wounds.

Corroborative evidence was noted for Ranson's finding regarding the growth of medullated nerve fibers across cicatricial tissue in the nervous system.

LITERATURE CITED

BURROWS, MONTROSE T.   1911   The growth of tissues of the chick embryo out-
    side the animal body, with special reference to the nervous system.
    Jour. Exp. Zoöl., vol. 10, no. 1, pp. 63–83.
DEL CONTE, G.   1907   Einpfanzungen von Embryonalem Gewebe im Gehirn.
    Ziegler's Beitrage zur pathol. Anat. u. z. allg. Pathol., Bd. 42, H. 1,
    S. 193–201.
GREENMAN, M. J.   1916   Regeneration of peripheral nerves.   Proceed. Phila.
    Neurolog. Soc., Journal of Nervous and Mental Diseases, vol. 43, no.
    1, pp. 62–67.
HARRISON, ROSS GRANVILLE   1907   Observations on the living developing nerve
    fiber.   Anat. Rec., vol. 1, no. 5, pp. 116–118.
LEWIS, WARREN H. AND LEWIS, MARGARET REED   1912   The cultivation of
    sympathetic nerves from the intestine of chick embryos in saline
    solutions.   Anat. Rec., vol. 6, no. 1, pp. 7–32.
RANSON, S. WALTER   1903   On the medullated nerve fibers crossing the site of
    lesions in the brain of the white rat.   Jour. Comp. Neur., vol. 13,
    no. 3, pp. 185–207.
    1909   Transplantation of the spinal ganglion into the brain.   Quar-
    terly Bull. Northwestern Univ. Med. School, pp. 1–4.
SALTYKOW, S.   1905   Versuche über Gehirnplantation.   Archiv f. Psychiat. u.
    Nervenkrankh.   Bd. 40, H. 2, S. 329–388.
THOMPSON, W. GILMAN   1890   Successful brain grafting.   New York Medical
    Journal.

SUBJECT AND AUTHOR INDEX

12810